# Sexual conflict over parental care in penduline tits

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*Award date:*
2009

*Awarding institution:*
University of Bath

# SEXUAL CONFLICT OVER PARENTAL CARE IN PENDULINE TITS

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Biology and Biochemistry

June 2009

# Contents

**CONCLUSIONS AND FUTURE DIRECTIONS**

# Acknowledgements

During my undergraduate studies biology at the University of Groningen, The Netherlands, I sometimes wondered whether a PhD project would really be suitable for me. Like many, I wondered if working on one study species for some four years would perhaps not be very exciting. Once I embarked on my PhD though, it soon turned out it would not be difficult to keep myself amused. Besides being lucky enough to be able to work on one of the most exciting species within research concerning sexual conflict and breeding systems and to have the privilege of living in the world heritage city of Bath, a substantial contribution to keep me happy throughout the past four years came from the fantastic bunch of the people that surrounded me. They have all been very supportive and have made life for the past few years a great deal more enjoyable.

First and foremost, I have very much enjoyed the privilege to work with two experts in the fields of sexual conflict and cooperation in breeding systems, my supervisors **Tamás Székely** and **Jan Komdeur**. I am very grateful to Tamás, first of all for inviting me to apply for a PhD at Bath after my MSc research project on penduline tits. I have learned an enormous amount from him. He has been extremely supportive throughout my PhD. His enthusiasm for the work we were carrying out has stimulated me a lot and never left me short of ideas where to go next. The opportunities for me to travel and explore new sites and penduline tit populations are also gratefully appreciated. When many attendants at one of the penduline tit meetings giggled at the idea of exploring penduline tits in Kazakhstan and China next year, Tamás was convinced it would be a worthwhile expedition and pursued the idea. It turned out to be a fantastic basis for future comparative research on penduline tits. I am also very grateful for being so accessible when he was in Bath (and not chasing plovers and students in some remote corner of the planet), despite the large number of projects he had to coordinate. The many chats we have had in his office have been enjoyable and at the same time very productive. It never took long to get back with useful and constructive comments on the work I put together, which I know is something not always easy to achieve, yet something extremely useful to get from a supervisor. We all know Tamás does have his grumpy days, but this is generously compensated for by a good and vibrant sense of humour.

I am very grateful to Jan for asking me to do my second MSc research project on penduline tits in 2004. I have learned a lot in that project about the fieldwork and important concepts in behavioural ecology and it thus formed an excellent basis from

which to start my PhD. Throughout my PhD Jan has also always been very supportive and easily accessible. The many comments he has provided on the various drafts of the chapters in my thesis, have helped enormously to improve the manuscripts. His and Tamás' comments were often very different and, although sometimes contrasting, they usually complemented each other very well. Furthermore, it has never taken Jan much longer than a day or so to find a suitable student from Groningen to carry out a project on penduline tits and help us to collect the necessary field data. Jan's good mood and hassle-free approach towards any issue we encountered made it a great pleasure to work with him. I appreciated that whenever I came over to visit Groningen there has always been a desk ready for me, and I could always rest assured that I could leave again having achieved everything I came for. The very different approach between the Animal Ecology Group in Groningen and Tamás' Biodiversity lab in Bath to tackle research questions have stimulated me to try and find the right balance.

Special thanks must also go to **Ákos Pogány**. It has been a great pleasure to work with him in the field. The long talks we have had about various issues have definitely contributed to the success of our penduline tit project. His skills to organise projects and develop field methods have been crucial to the success of our field work in South Africa and Asia. And also the more informal chats we have had in various pubs in Hungary, South Africa and England have also always been most enjoyable and may or may not have contributed to the success of our project.

I am thankful to **István Szentirmai** for being such a great company during my first field season on penduline tits in Fehértó in 2004 and during several meetings later on. István also deserves the credits for developing most of the field methods.

**Lídia Mészáros** also made sure our time in the field in Hungary has always been very enjoyable. She has been a great person to collaborate with and, like Ákos and István, became a good friend. All three of them were also always kind enough to offer me the opportunity to let me stay at their place at any time. Lídia also dealt with most of the practicalities in Fehértó.

The help of all the students and field assistants that have participated in our project, and of the people that have collaborated in or have facilitated to start up projects at various localities is also acknowledged with gratitude: **Joris van Alphen, Sander Bot, Dušan Brinkhuizen, Natalino Cuti, Johan van der Dennen, Richard ffrench-Constant, Per Ericson, Zsofia Gergely, Rob Gordijn, Martijn Hammers, Péter Horváth, Martin Irestedt, Corinne Jeffs, Evelien Jongepier, Arno wa**

**Kangeri, Penn Lloyd, Bruno Massa, Emily Mockford, Arjen Pilon, Sergey Sklyarenko, Rafaella Turco, Machiel Valkenburg, Marco van der Velde, Vera Voronova, Arend Wassink, Franjo Weissing** and **Xutong Yang**. Without them we would have been able to collect considerably less data, and some preliminary results, important for the further development of projects, would have been produced at a much later stage or not at all. The members of the biodiversity lab in Bath, a great, international bunch of people, also deserve a great expression of gratitude. They not only helped to create a stimulating working environment, but many of them also became good friends without whom life in academia would have been much less enjoyable: **Araceli Argüelles Ticó, Monif Al-Rashidi, Fiona Burns, John Burnside, Richard James, Gabriel Garcia Peña, Freya Harrison, András Kosztolányi, Clemens Küpper, Peter Long, Mark O'Connell, Valérie Olson, Alejandro Serrano Meneses, James St Clair** and **Richard Young**.

I am grateful to **Nick Davies** for taking up the external examination of my thesis and to **Steve Dorus** for doing the same as my internal examiner.

I also thank the organisations and committees that have been responsible for the funding of the various projects, in particular the University of Bath for providing me with a University Studentship. All other financial support is recognised in the acknowledgements of the separate chapters in my thesis. I am grateful to the Kiskunság National Park and Szegedfish Ltd. for providing permission to conduct fieldwork in Hungary and to ESKOM for doing likewise in South Africa.

I am also greatly indebted to my parents, **Peter** and **Tineke**, who have always generously supported, but never pushed, me to work at the limits of my abilities, even if this meant that, after my brother Ronald moved to Ireland, I would leave The Netherlands too. Everything I have achieved and the privileged life I have been living over the past 28 years would not have been possible without their immeasurable support and understanding.

Finally, special thanks go to the numerous people I forgot to or could not mention here. There are far too many, but each one of them has made a significant contribution to the success of our project, to my understanding of the penduline tit's natural history and behavioural ecology, and/or has helped me to enjoy my work, wherever on the planet I was. I am very grateful to all of them. I do hope to be able to stay in touch with most of the people mentioned above and hope to be able to continue to successfully collaborate with you in this exciting project.

After a very enjoyable PhD, as always in science, we have generated more questions than answers. But after four years I am still increasingly enjoying working with penduline tits. And the people that are associated with this project, whether in the field, in the lab, or at home, guarantee that a project like ours would be enjoyable for at least another forty years.

## Summary

Sexual conflict, the different interests of males and females over reproduction, is a potent evolutionary force. Here I investigate sexual conflict in the context of parental care by focussing on two questions: (i) which behavioural, morphological and environmental traits influence the parents' decision to care for the brood or desert? (ii) How does sexual conflict influence the evolution of behaviour and morphology? I investigate both questions using a small, polygamous passerine bird, the Eurasian penduline tit *Remiz pendulinus*, which exhibits intense sexual conflict over parental care such that either the male, the female or both parents desert the clutch. Using detailed behavioural observations during the crucial few days preceding desertion, I argue that it may be in the parents' best interest to conceal their intention to care for (or desert) their brood. The rapid resulting process that leads to variable parental care resembles a coordination game in which either parent may desert first. I developed a game-theoretical model that suggests that a key to resolving the conflict between parents is the sex difference in reproductive payoffs for given parental care strategies, rather than differences in parental quality per se. Environmental variables (e.g. food availability and mating opportunities) seem only subsidiary in the decision-making process of parents. My final chapter explores ramifications of sexual conflict at an evolutionary timescale. By comparing the highly polygamous Eurasian penduline tit with the monogamous Cape penduline tit, I show that morphological and behavioural differences between these two species are consistent with predictions of sexual conflict theory. During my PhD I also identified that there is considerable variation in breeding systems within different species of penduline tits. I argue that by studying these systems new insights will emerge into (i) the drivers of breeding systems, and (ii) neural and genomic traits that underlie breeding system evolution.

# CHAPTER I

## INTRODUCTION
## SEXUAL CONFLICT AND COOPERATION

**René E. van Dijk**

The life of animals is rife with strategic decisions. Perhaps the most important strategies to adopt are those concerning reproduction. The concepts of sexual conflict and cooperation are used to describe and understand how breeding systems evolve, and what the evolutionary implications of reproductive strategies (for instance mating behaviour and parental decisions) are. Although the two concepts can be considered as describing contrasting strategies and are often investigated separately, in reality they should be considered as one framework to understand the full range of breeding systems found in nature.

## SEXUAL CONFLICT

Sexual conflict, i.e. a conflict between the evolutionary interests of individuals of the two sexes (Parker 1979) is ubiquitous in nature (Arnqvist & Rowe 2005). Indeed, there are only two scenarios where sexual conflict can be expected to be absent, firstly in the case of full and lifelong monogamy, and secondly when an animal only breeds once in its entire lifetime ('semelparity'). Both of these scenarios are rare in nature (Lessells 2006, *Chapter II*). Although widespread, the extent to which sexual conflict is exhibited varies widely. Intense sexual conflict can result in extreme evolutionary outcomes (Chapman et al. 2003; Arnqvist & Rowe 2005; Pizzari & Bonduriansky 2009). Some of the striking examples stem from pre-zygotic sexual conflict (sexual conflict over mating). In bed bugs *Cimex lectularius*, for instance, the conflict over mating rates results in traumatic insemination of females, when the male inseminates into the female body cavity. This behaviour leads to an enhanced fertilization success for the male, but it is harmful for the female and it impairs her reproductive output (Stutt & Siva-Jothy 2001). Other striking examples are the variety of so-called copulatory plugs, which occur across the animal kingdom and prevent the female from successfully mating again after copulating with a male (e.g. Fiumera et al. 2006; Moreira et al. 2006; Kuntner et al. 2009). These examples illustrate that reproduction is not always a cooperative venture.

It is not only the frequency of mating over which a conflict between the sexes exists. Once fertilisation has taken place, each parent will try to minimise the costs of reproduction whilst gaining a maximum benefit. Both parents will benefit from having successfully raised offspring, yet it is in the best interest of both to shift the costs of parental care to their partner ('post-zygotic sexual conflict' or 'sexual conflict over parental care'; Trivers 1972; Davies 1992; Balshine et al. 2002; Royle et al. 2002; Houston et al. 2005). How parents resolve this conflict over care and

which variables may influence the negotiations over parental care, are the issues I address in this thesis. A general introduction to the concept of sexual conflict over parental care is outlined in detail in *Chapter II*.

## COOPERATION

A definition of cooperation is context dependent, although it should generally be in the form of individual strategies describing the cooperation, cooperative investment and the cooperative returns (Noë 2006; West et al. 2007; Wenseleers et al. 2009). Given the ubiquity of conflict as described above, the existence of cooperation between individuals seems evolutionarily counterintuitive (cf. 'selfish genes', Dawkins 1976). Nevertheless, cooperation can be observed at various levels, from genes to societies, in many different taxa, and interestingly also closely related species may show a large variation in the levels of cooperation exhibited (Mank & Avise 2006; Thomas et al. 2007; Kroll & Shogren 2008; Maclean & Brandon 2008; van Dijk, RE, Pogány, Á, & Székely, T. unpubl. data).

Cooperation receives an enormous attention from scientists in a very diverse research community spanning from neurobiology to political sciences (Pennisi 2005). However, the exact mechanisms (proximate causes) that confer direct or indirect fitness benefits (ultimate causes) on cooperation still need to be explored, as does the structure of the cost and benefit functions (i.e. payoffs) of cooperation. Research so far relied for a large part on game-theoretical models, which have been criticised to be restrictive and somehow unrealistic (Noë 2006). One of the most influential game-theoretic models describing levels of cooperation is the Prisoner's Dilemma (Axelrod & Hamilton 1981; Nowak & May 1992; Heinsohn & Packer 1995; McNamara et al. 2004; McNamara & Weissing 2009). In the Prisoner's Dilemma the situation is described where the tendency of individuals to defect towards cooperators results in the breakdown of cooperation. To date few empirical examples have been published providing support for this influential model (see *Chapter V*). Therefore, it seems unlikely to find a general, realistic model that explains why animals cooperate; the appropriate models likely depend on which type of cooperation is being investigated.

## BREEDING SYSTEMS

In order to reproduce, the members of a pair have to cooperate. However, beyond this minimum level of required cooperation, the selfish benefits for each individual

will become paramount. The extent to which this is exhibited is likely determined by the social and non-social environment (*Chapter II*). Food availability, for instance, is often associated with the level of conflict or cooperation in the breeding system (Emlen & Oring 1977; Erckmann 1983; Davies & Lundberg 1984; Davies 1991; Davies 1992; Wiklund et al. 2001; Eldegard & Sonerud 2009). In a monophyletic group of acrocephaline warblers, for instance, habitats with abundant resources were associated with the occurrence of polygyny and male promiscuity, whereas poor habitats were characterised by monogamy and the occurrence of helpers at the nest (Leisler et al. 2002). It has been argued that the different reproductive payoffs for males and females in conjunction with the environment drive breeding system evolution (Davies & Lundberg 1984; Davies 1992; Székely et al. 2006; McGraw et al. 2009; *Chapter V*). If the environment promotes the emergence of sexual conflict, uniparental care and polygamy may be the result, whereas if there are environmental constraints, cooperation between the parents may be the most beneficial strategy. Subsequently, the various levels of polygamy that are associated with the different breeding systems are related to the variance in reproductive success upon which sexual selection may act. This is then one possible route along which sexual conflict and cooperation may drive the evolution of morphology and behaviour of animals (Wilson et al. 2001; Arnqvist & Rowe 2002a; Chapman et al. 2003; Pizzari & Bonduriansky 2009) and facilitate speciation (Arnqvist et al. 2000; Wilkinson & Birge 2009).

In this thesis I adopt the view that cooperation and conflict (in particular sexual conflict) are two sides of the same coin. Much work on animal cooperation refers to single interactions between two individuals (or an individual and an experimental apparatus) (Brosnan & de Waal 2003; West et al. 2006; Jensen et al. 2007; Dreber et al. 2008; Antal et al. 2009; Helbing & Yu 2009). Jensen et al. (2007), for instance, describe how two chimpanzees, *Pan troglodytes*, may or may not cooperate with each other to obtain food items from an experimental apparatus by pulling one rope each. They show, in an ultimatum game, how chimpanzees are insensitive to fairness, thus distinguishing them from social organisations in humans. But also the interactions between members of an extended family (i.e. cooperative breeding, Komdeur 1992; Clutton-Brock et al. 2002; Burkart et al. 2007; Hatchwell 2007; Sharp et al. 2008) receives a large amount of attention from researchers. In this thesis my focus is on breeding systems, and I argue that sexual conflict and

cooperation are two key concepts, and both are needed to reveal the diversity of breeding systems.

## SEXUAL CONFLICT AND COOPERATION IN PENDULINE TITS

The subfamily of penduline tits (Remizinae, small passerine birds with a body mass of 6-12g, that build a characteristic complex, domed nest (Cramp et al. 1993; Harrap & Quinn 1996; Madge 2008)) is an excellent avian taxon to investigate the potential causes and implications of sexual conflict and cooperation. Penduline tits exhibit diverse breeding systems ranging from uniparental care by either sex with a substantial proportion of nests deserted by both parents (Eurasian penduline tit, *Remiz pendulinus*), uniparental care by the female (Chinese penduline tit, *Remiz consobrinus*), to biparental care and facultative cooperative breeding (Cape penduline tit, *Anthoscopus minutus*, Harrap & Quinn 1996; Dean 2005; van Dijk, RE, Pogány, Á, & Székely, T. unpubl. data, see also *Chapter X; Appendix IV*). Remizinae breed throughout Eurasia and Africa in various habitats from reed marshes with abundant resources through to relatively poor, arid semi-deserts (Harrap & Quinn 1996; Madge 2008; *Chapter X*).

## OUTLINE OF THE THESIS

In this thesis I focus on the best studied species within the subfamily, the Eurasian penduline tit, *Remiz pendulinus*. This species is characterised by intense sexual conflict over parental care: both parents appear to benefit from deserting rather than caring for the offspring in terms of reproductive success (Szentirmai et al. 2007; *Chapter V*). Only one parent (either the male at 5-20% of nests, or the female at 45-70% of nests) incubates the eggs and rears the young. Interestingly, these percentages do not add up to 100%, since some 30-40% of nests is deserted by both parents possibly due to the intense sexual conflict between parents (Arnqvist & Rowe 2005; Szentirmai et al. 2007; this thesis). After desertion, many parents remate so that both males and females may have up to six mates in a given breeding season (Persson & Öhrström 1989; Szentirmai et al. 2007; this thesis). Biparental care has rarely been reported in Eurasian penduline tits (see Schönfeld 1994 and *Chapter VIII*). By calculating the expected versus observed frequencies of different care types in the population, McNamara et al. (2002) concluded that the decisions of males and females (care or desert) are unlikely independent.

*Chapters I – II* introduce the main concepts and issues considered in the thesis, review post-fertilisation reproductive strategies, and discuss how animals resolve sexual conflict over care, the implications thereof and the mechanisms of parental decisions.

*Chapters III – VI* focus on the question of how male and female parents interact and decide whether to care for the offspring or to desert the clutch. Using behavioural observations and time-lapsed photos at the nest, I investigate the process of desertion during the crucial few days before desertion takes place, and analyse whether parents signal their intention to desert (*Chapters III – IV*). I then develop a game-theoretical model to understand the patterns of parental care in Eurasian penduline tit and ask to what extent the reproductive strategies are predicted by the payoffs from caring and deserting (*Chapter V*). I also investigate what causes the apparent sex difference in reproductive payoffs by testing whether females provide better care than the males (*Chapter VI*).

*Chapters VII and VIII* show how the decision about parental care may be influenced by the environment. I test how the structure of the habitat (a proxy for the availability of food and nest material, *Chapter VII*) and breeding density (likely associated with a number of social variables such as the availability of mates and competitive interactions, *Chapter VIII*), predict patterns of parental care in different populations of penduline tits.

*Chapter IX* compares the breeding systems of Eurasian penduline tit and Cape penduline tits, a socially monogamous species. I compare the two species to test a priori predictions of sexual conflict theory and investigate the potential impact sexual conflict over care may have on the evolution of morphological and behavioural traits. Additionally, I test whether females mated to attractive mates may incur a cost in terms of reproductive output, as predicted by sexual conflict theory.

*Chapter X* provides a synthesis of my major results and discusses them in a more general framework. Potential future avenues are also proposed here.

# CHAPTER II

# POST-FERTILISATION REPRODUCTIVE STRATEGIES

**René E. van Dijk & Tamás Székely**

*Encyclopedia of Life Sciences (ELS)* (2008)

*Authors' contributions*

**REvD**: study design, literature review, manuscript preparation

**TS**: study design, literature review, manuscript improvement

## ARTICLE DEFINITION

Animals have diverse strategies that improve the success of their offspring after mating (i.e. post-fertilisation reproductive strategies); the most common ones are parental care and offspring provisioning. The type, mode and duration of parental care exhibited by males and females depend on social and non-social environment, and on genetic and phylogenetic constraints. We overview five rapidly developing areas of parental care research, and conclude that sexual conflict between parents, social interactions and environmental conditions play important roles in determining post-fertilisation reproductive strategies.

## INTRODUCTION

Post-fertilisation reproductive strategies have a broad remit, and may include aspects of an individual's behaviour toward its offspring, mate, and other members of its social environment. In this review, however, we focus on the interactions between parents and their offspring, since this research is rapidly advancing and integrate results from various disciplines including genetics, behavioural ecology and phylogenetics. We focus on families, and view family life as rife with conflicts. Within a family, there may be (i) a conflict between the male and the female parents (sexual conflict) since the benefit, in terms of fitness through the offspring is shared by both parents, whereas each parent pays the cost of care provisioning itself (Trivers 1972; Balshine et al. 2002), (ii) parent-offspring conflict since the offspring's best interest is to take as much resource as they can get, whereas the parents' interest is to balance the resources between their current offspring and those they may produce in future (Trivers 1974; Lessells & Parker 1999), and (iii) conflict between siblings, since each individual offspring is expected to promote its own existence over that of its brothers and sisters (Mock & Parker 1997). These conflicts may reduce the optimum reproductive output of the families (Parker et al. 2002). The outcome of these conflicts will be influenced by the social and non-social environment (Kilner 2002). In this review we focus on the core family, and do not discuss extended families such as in cooperative breeders and in eusocial insects (Keller & Chapuisat 2002).

In this review we focus on conflicts between parents over care (Houston et al. 2005), or, as frequently – and incorrectly – referred to as 'parental investment' (Clutton-Brock 1991). The term 'parental investment' is often incorrect, since many studies have referred to it as the energy 'expended' on the offspring by the parents. However, Trivers (1972) defined it as the cost in terms of future reproduction. The latter is extremely hard to measure in nature. A conflict between parents over care seems inevitable, and likely to have a profound influence on post-fertilization reproductive strategies. The optimal amount of care by a parent is often less than the care that it would prefer from its mate, unless the animals only breed once in their lifetime ('semelparity'), or there is full and lifelong monogamy between a pair, which rarely occurs in nature (Lessells 2006). Yet, a male and a female have to cooperate, at least to some extent, in order to reproduce. The environment is likely a major determinant of the manifestation of the conflict, since it influences how much an individual parent may gain by reducing its parental care and saving resources for

future matings (i.e. effects of social environment), and how much the parents need to care in order to successfully raise the offspring in a given non-social environment. One parent may completely withhold care: leave its partner and offspring ('offspring desertion') and save its resources to breed in future.

Sexual conflict over care may be resolved over evolutionary time, or on a contemporary time scale. An evolutionary solution, e.g. 'female-only care', may emerge if one sex is preadapted to provide parental care. Only female mammals, for instance, have mammary glands, so males cannot suckle young. Since suckling is an essential part of offspring care, female mammals cannot shift all care provisioning to the males. In contemporary populations, however, conflict resolution may be plastic, so that the split of care provisioning between males and females can vary between individuals and populations.

Parental care is female-biased in many organisms, although various ecological conditions underlie a suite of phylogenetic transitions in parental care to male-biased care or to biparental care in a wide variety of vertebrates (Reynolds et al. 2002). Phylogenetic comparative analyses, provide unique insights into the evolution of parental care on an evolutionary timescale, whereas studies of contemporary populations in the field or laboratory allow us to test which variables are important in moulding a species' breeding system. For example, shorebirds and relatives (Charadriiformes) exhibit different levels of male care and female care, including full care provisioning by both parents until the young fledge. A major component of this interspecific variation is offspring demand; species that have demanding young (i.e. semiprecocial chicks that beg food from their parents) invariably exhibit biparental care, whereas shorebirds with less demanding young (i.e. precocial chicks that find food for themselves) may have uniparental care by the male or the female. Within this phylogenetic constraint brood care by males and females can be variable: at low population density female Kentish plovers desert the brood to seek a new mate, whereas at high population density the female stays with the brood and help the male to defend the chicks from neighbours (Kosztolányi et al. 2006).

## WHICH SEX SHOULD PROVIDE CARE?

Biparental care is rare in animals, apart from cichlid fishes, birds, and primates (Clutton-Brock 1991; Reynolds et al. 2002). In many vertebrates there is a female bias in parental care (Queller 1997). Phenotypic plasticity, the ability to adopt various strategies under variable conditions, allows individuals to adopt the strategy

that optimises their fitness in various social and non-social environments leading to diverse breeding systems within a population. For example, a high abundance of food, low predation rates, and a relatively mild climate, may allow one parent to raise offspring successfully. In such a scenario, one parent decreases its parental care and may ultimately desert, leaving the partner to care for the offspring. The deserting parent saves the costs of care in terms of time and energy, and these in turn may then be invested in a new batch of offspring (Fig. 2.1). For instance, in the Eurasian penduline tit, *Remiz pendulinus* (Fig. 2.2) parental behaviour is phenotypically plastic: deserting the nest and mate increases the reproductive success for both the male and the female. Desertion, however, is harmful for the deserting parents' mate, since it reduces the reproductive success of its mate (Szentirmai et al. 2007). As a result of intense sexual conflict, a range of breeding systems are observed within a population: some nests are cared for by one parent only (either male or female) and about one third of clutches is deserted by both parents. In biparentally deserted nests all offspring die - a substantial cost of antagonistic interests of males and females.

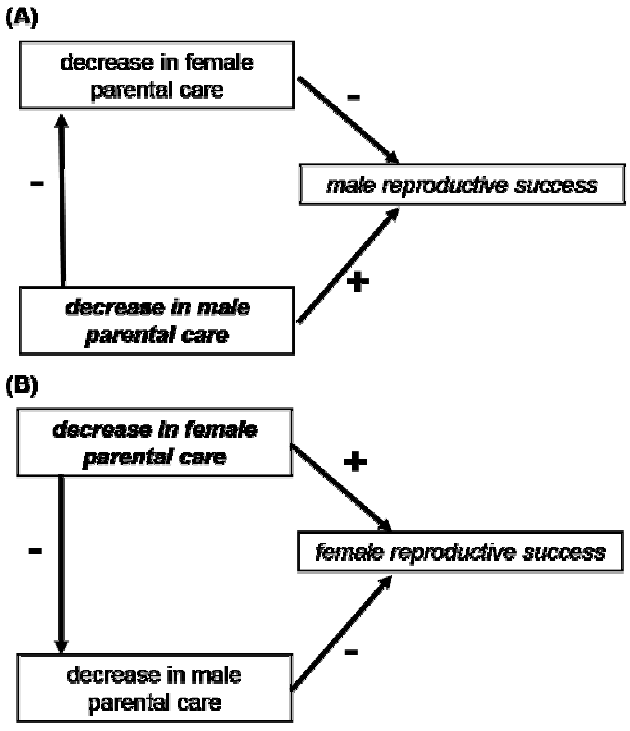


**Figure 2.1** Central to the question how much care a parent should provide is the trade-off between current and future reproduction. In general, if a male has the opportunity to decrease parental care (A) this may constrain the possibility for his partner to do so, and the female may even compensate for the lack of care by the male. By decreasing paternal care, the male may enhance his own reproductive output by acquiring multiple mates, or by enhancing his own longevity, thus enhancing his future reproductive output. If the female, on the other hand, decreases parental care, this will negatively affect the male's reproductive output. If the female has the opportunity to lower parental care (B), the effects are a mirror image. An increase in parental care by either parent is expected to reduce its future reproductive output. The focal sex (A: male, B: female) is in italics in both diagrams. (see Szentirmai et al. 2007)

**Figure 2.2** The Eurasian penduline tit, *Remiz pendulinus*, has an extremely variable breeding system among birds: incubation and feeding of nestlings is carried out by either the male, or the female, whereas about 30-40% of nests is deserted by both parents. This diverse breeding system appears to be driven by intense sexual conflict over care. (Photograph R.E. van Dijk)

Which parent may desert, however, is likely to depend on the social environment (such as the number of available mates) that influences benefits of desertion. If remating opportunities are low, the deserting parent may not be able to start a new brood, so that it might be better off assisting its partner in raising the offspring. In addition, if mating opportunities are limited for males, then females may demand paternal care in return for mating access. Game-theoretic models revealed important insights about the interactions between pairs and the rest of the population (Alonzo & Warner 2000; McNamara et al. 2000), although it is not yet known how the feedbacks between breeding and non-breeding population members work out in nature.

## HOW MUCH CARE SHOULD PARENTS PROVIDE?

*Brood size*

In many animals the offspring require little or no parental care. These include species that exhibit precociality (the offspring leaves the nest after hatching, often still guarded and partly fed by the parents), and inter- and intraspecific brood parasitism (the offspring is fully raised by an alloparent; e.g. common cuckoo, *Cuculus canorus*, and common goldeneye, *Bucephala clangula*, respectively) found among a variety of taxa including birds, fish and insects. Generally, however, parents pay a cost of raising offspring (Fig. 2.1), and studies have pointed out various costs of reproduction in terms of future offspring for a wide range of taxa. Parents are thus

likely to trade-off the amount of care they invest in a current brood against the care in future offspring (Fig. 2.1).

One solution to this trade-off is to optimise brood size. Larger broods will result in a higher reproductive output, which may come at a cost in future. Also, care per offspring generally declines with larger broods, which limits the survival of the offspring. Furthermore, larger broods not only mean greater demands on the parents' provisioning, but brood size also commonly affects brood defence, since it will be more worthwhile for the parents to defend a larger brood than a small brood (Clutton-Brock 1991).

The optimal brood size may be confounded by a multitude of variables. If food availability, for instance, is unpredictable, the optimal brood size is likely too. One strategy to get around unpredictability is facultative siblicide (Mock & Parker 1997). Under favourable conditions the parents raise multiple offspring within a brood, whereas if food is scarce the parents deliver a limited amount of food and competition between siblings may reach lethal levels.

Sibling competition may also be influenced by maternal effects. Mothers may allocate different levels of food resources or hormones such as testosterone to their offspring. The differential allocation of food or deposition of testosterone can compensate for a delay in growth of later born offspring, enhancing their ability to compete for food with the earlier born and thus often larger and more competitive siblings (Kilner 2002).

*Brood sex ratio*

Parents can also adjust the amount of care in relation to the sex ratio of the brood. The costs of raising sons or daughters may be different, and may thus have a differential effect on, for instance, survival of the parents. In sexually size dimorphic species the offspring of one sex may be more costly to raise than offspring of the other, albeit sexual size difference may provide a poor estimate of parental care, due to sexual differences in body composition and advanced growth (Clutton-Brock 1991). Nevertheless, the fitness of the offspring, and thus that of the parents, may vary with offspring sex. In polygamous species, for instance, parents of higher quality may benefit more from investing in offspring of the sex that may attract multiple mates, since the breeding success of the sex with the most variable reproductive success will depend more on adult size and attractiveness (Trivers & Willard 1973). The differential effect of parental care on the fitness of sons and

daughters probably varies widely, although the variance in reproductive success tends to be higher in males than in females (Clutton-Brock 1991). Additionally, if the offspring is philopatric and cooperates with the parents after independence, the costs and benefits of care for offspring remains hard to estimate (Clutton-Brock 1991).

*Parentage and parental care*

Certainty of parentage, i.e. the genetic share a parent holds in its offspring, is expected to influence the amount of parental care, and thus may differ between males and females. Males often increase their reproductive success from engaging in extra-pair copulations, and they guard their mate to ensure that they will not be cuckolded in the brood they share with their social partner. Females, however, may benefit from extra-pair young in a variety of ways: by having more genetically diverse offspring, gaining direct benefits such as access to territories of several males, or having multiple males contributing to parental care. The outcome of the conflicts between sexes, in conjunction with the certainty of paternity, may predispose females to care more often than males (Queller 1997).

Empirical studies support the conflicting interests of sexes over parentage; for instance male blue-footed boobies, *Sula nebouxii*, adjust their willingness to care based on certainty over paternity. However, other studies did not find relationships between parentage and paternal care, and the exact relation is not clear and needs further investigation (Sheldon 2002).

## DO PARENTS NEGOTIATE OVER CARE PROVISIONING?

The optimal level of provisioning by one parent likely depends on the behaviour its mate. One approach to investigate the optimal level of care if to use discrete behaviours (care or desert) in desertion games, or a continuous adjustment of care ('parental effort game'). These games may ultimately lead to full cooperation between the parents, or result in biparental desertion (Szentirmai et al. 2007).

The best strategy predicted by these game-theoretic models may depend on various variables, including the behaviour of other members in the population (McNamara et al. 2000), and the attractiveness of the partner. With regards to attractiveness, however, observations and experiments provided contrasting results, since sometimes attractive males increased their care provisioning (e.g. in northern cardinal, *Cardinalis cardinalis*, (Linville et al. 1998), consistent with the prediction of the good parent hypothesis), whereas in other species attractive males reduced

their parental provisioning (e.g. in zebra finch, *Taeniopygia guttata*, (Burley 1988), consistent with the prediction of the differential allocation hypothesis). A limitation of existing studies, however, is that they assume that attractiveness can only influence male behaviour and female response to male quality; in reality, a two-way process is likely in which female attractiveness and male response also matter.

The currencies underlying such state-dependent decisions may be involved in a process of manipulation of the partner. A parent may, for instance, handicap itself so as to manipulate the partner to work harder (Smith & Härdling 2000; Barta et al. 2002). The idea of partner manipulation is consistent with the logic of sexual conflict theory (Lessells 2006), although as yet, there is little direct evidence on partner's manipulation in the parental care context, and thus experiments are needed to test these theoretical ideas.

Mates may not decide independently over parental care. In the Galilee St Peter's fish, *Sarotherodon galilaeus*, for instance, both parents circle over the eggs after fertilization, before one picks up the eggs for mouth incubation. During circling the parents may monitor each other, and possibly negotiate over care. We argue that signalling an intention to reject care provisioning (and deserting) may not be evolutionarily stable, because this would allow the mate to retaliate by deserting as a pre-emptive strike.

Should parents compensate for the lack of care by their partner (Fig. 2.1)? The parental effort game (Houston & Davies 1985) predicts that only partial compensation should lead to biparental care being an evolutionarily stable strategy, whereas full or over-compensation is likely to lead to one parent doing all the work. Empirical studies provided various results from no compensation to full compensation (Houston et al. 2005). It appears that a reason for the different outcomes of experiments is the amount of information available to the parents about the demands of the brood (McNamara et al. 1999; Johnstone & Hinde 2006).

## PARENTAL BEHAVIOUR: NATURE AND NURTURE

Evolution by natural selection can only occur if a trait has additive genetic variation and is genetically heritable, i.e. a proportion of phenotypic variation in a population is attributable to genetic variation among individuals. Studies suggest that parental behaviour is consistent for a given parent (i.e. repeatable), and may have a low, albeit significant, heritable component. Heritability of brood feeding rates, for instance, varies between no heritability and high heritability in birds, with males showing

higher heritability than females (MacColl & Hatchwell 2003; Nakagawa et al. 2007), and helping relatives to rear young has a heritable component in western bluebird, *Sialia mexicana* (Charmantier et al. 2007). Note that these heritability estimates take into account some of the apparent environmental effects such as the number of chicks in a nest. It is usually assumed that the correlations between parental behaviour and the behaviour of their offspring when they grow up are due to shared genes, however, this may not be strictly true because behaviour can be learnt from parents, passed on non-genetically from relatives via social inheritance, or due to maternal effects, e.g. amount of hormones deposited in the eggs and social environment during rearing may imprint offspring behaviour.

Experimental manipulations (removals, handicapping, see above) show that parents can adjust parental responses to social and non-social environment; therefore, it is very unlikely that parental care is fully determined by genes. An elegant study conclusively supporting the influence of environment on parental behaviour was carried out by translocating breeding Seychelles warbler, *Acrocephalus sechellensis*, from one island where the population was at carrying capacity and exhibited high incidence of helping behaviour into a new island devoid of warblers (Komdeur et al. 1995). Initially, no helping behaviour was observed on the new island, but as the territories became saturated helping appeared and became common.

## HORMONAL MECHANISMS

Parenting is an entire repertoire of behaviours, not a single behaviour (Adkins-Regan 2005). Parenting strategies are diverse, and the differences between closely related species suggest neither parental behaviour itself nor the underlying mechanisms are homologous. Even though the physiology and neuroanatomy of parental behaviour have been well studied in rats, rabbits, and sheep, we don't know the relevance of their findings to other mammals or to non-mammalian taxa.

Hormones do not 'cause' parental behaviour, rather they prime the organisms to carry out certain tasks and modulate behaviour; for instance experience in giving birth and rearing young, sensory stimuli from the pups, and appropriate hormone levels may all be needed to elicit maternal behaviour in Norway rat. In female rats, hormones of pregnancy, particularly oestrogen and progesterone, prime the brain to promote acceptance of offspring (Young & Insel 2002). This activation is focussed on specific areas of the brain (including the medial preoptic area), and increase the sensitivity of receptors to prolactin and oxytocin. The precise mechanism by which

prolactin and oxytocin lead to maternal actions are not known, although studies of knockout mice show that females lacking functional prolactin receptor fail to retrieve pups whereas females having two normal copies of the prolactin receptor gene exhibit normal pup retrieval behaviour.

In female voles oxytocin elicits maternal behaviour as in mice and rats, although oxytocin also plays a prominent role in regulating the capacity of females to form a pair bond with her mate (McGraw *et al.* 2009). In socially monogamous prairie voles, *Microtus ochrogaster*, females display a partner preference after mating and cohabitation with a male. Oxytocin release during copulation or infusion of oxytocin into the brain during cohabitation with a male accelerates the development of partner preferences.

Although paternal care is more common in teleost fish and birds, it is probably best studied in small mammals, particularly in voles. It would be convenient to think of male care as the antagonist modulation in females, but this does not seem to be the case. Studies in prairie voles suggest vasopressin, a neuropeptide closely related to oxytocin, is important for paternal behaviour (Young & Insel 2002), since vasopressin injected into a males' brain increased paternal behaviour, whereas vasopressin antagonist decreased it. Although oxytocin and vasopressin are different neuropeptides, they only differ by two amino acids, and probably originated from a common ancestral gene. Oxytocin regulates female parenting and pair bonding behaviours while vasopressin appears to serve this role in males. Variation in the localization of oxytocin and vasopressin receptors - especially in regards to highly repetitive DNA sequence upstream of vasopressin-encoding receptor that is long in monogamous prairie voles but short in promiscuous meadow and mountain voles *Microtus montanus* (Hammock & Young 2005) - appear to contribute to differences between species and individuals in regard to social behaviour. Studies beyond these well-studied voled, however, in other species of voles and in primates found no consistent relationship between microsatellite length and mating system (Fink et al. 2006).

Studies of voles, rats, and mice suggest several important points. Firstly, steroids and peptides are more important for the onset of maternal behaviour than for its continued maintenance. Secondly, since several peptides, steroids and proteins are involved in regulation of maternal behaviour, it is futile to seek THE maternal hormone (Adkins-Regan 2005). Thirdly, it is important to move away from the one gene – one behaviour paradigm – to understand the cascade of cellular actions that

alter neural pathways which results in behavioural change (Young & Insel 2002). Finally, although oxytocin and vasopressin act in distinct brain regions and are involved in female and male parental behaviour, respectively, they act within the same brain circuit (McGraw et al. 2009). Therefore, females and males have evolved different parental care strategies due to different hormonal regulation. These may be accomplished by changing the responsiveness of the same neural circuit in the brain.

## CONCLUSIONS AND OUTLOOK

We draw three major conclusions from this overview. Firstly, we argued that conflict between parents has a major influence on care provisioning. However, cooperation should also influence care, and full cooperation between parents is important for raising young in many species. Therefore, an important aspect for future studies is to establish the relative roles of conflict and cooperation in parental care evolution. Secondly, experiments suggest that parental effort is not fixed: parents can adjust their effort if they need to. The outcomes of these experiments, however, are conflicting, and it is not understood why parents compensate in some species but not in others. Thirdly, social traits – such as post-fertilisation reproductive strategies – are influenced by both genetic and environmental effects. It would be naïve to assume one of these is 'more important' than the other, rather, we anticipate their interaction produces the diverse parental care systems we see today. Teasing apart these sophisticated interactions may signal a new aura, sociogenomics (Robinson et al. 2005). Finally, we note that parental care and breeding strategies of many animals – particularly tropical species – have not been studied in detail, and we anticipate major advances in natural history of post-fertilization reproductive strategies by discovering new species and investigating their breeding ecology. Given the predicted loss of a substantial number of species in the near future, the task of field ecologists, sociobiologists and behavioural geneticists to discover and understand biodiversity is more urgent than it has ever been before.

**Further Reading**

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# CHAPTER III

## SEXUAL CONFLICT OVER PARENTAL CARE IN PENDULINE TITS *REMIZ PENDULINUS*: THE PROCESS OF CLUTCH DESERTION

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*Ibis (2007) 149: 530-534*

*Authors' contributions*

**REvD**: study design, data collection, statistical analyses, manuscript preparation

**IS**: data collection, manuscript improvement

**JK**: study design, manuscript improvement

**TS**: study design, data collection, manuscript improvement

**ABSTRACT**

Do the two parents at a nest make simultaneous decisions whether to care for their offspring or to desert? If a single parent is sufficient for rearing young, one parent (typically, the male) may desert and reproduce with a new mate within the same breeding season, leaving the other parent with the brunt of care. Since each parent is expected to maximise its own reproductive success, the interests of the two parents do not necessarily coincide, and a sexual conflict over care may emerge. Here we investigate the process of clutch desertion in a small passerine bird, the Penduline Tit *Remiz pendulinus*. Among birds, this species has a remarkably variable breeding system, because a single parent (either the male or the female) may provide the full care of the young, whereas about 30% of clutches are abandoned by both parents. First, we show that biparental desertion occurs within a single day in 73.7% of the clutches (n = 14), whereas desertion decisions are sequential in 26.3% of the clutches (n = 5) (male first: 10.5% (n = 2); female first: 15.8% (n = 3); n = 19 clutches in total). Second, we observed the behaviour of both parents before desertion, and investigated whether desertion can be predicted from their behaviour. However, neither singing nor nest-building behaviour predicted whether the male or the female would desert. We therefore suggest that biparental desertion may be simultaneous by male and female in our population of Penduline Tits. Furthermore, the parents do not appear to signal their intention to desert their mate. We argue that the parents' interest may be actually to disguise their intention to desert.

## INTRODUCTION

How do parents decide which of them should provide care? To understand parental behaviour, we need to know the costs and benefits of caring and deserting (Clutton-Brock 1991; Székely et al. 1996), and the process of interactions between the parents (Houston & McNamara 1999). Whilst data on the costs and benefits of caring are gradually accumulating from several avian species (reviewed by Bart & Tornes 1989; Liker 1995; Houston et al. 2005), the understanding of parental interactions has lagged behind. Most models of parental care (e.g. Maynard Smith 1977; Houston & Davies 1985) assume that the parents make a single and simultaneous decision independent of their mate ('sealed bid', Royle et al. 2002). Theoretical analyses show that, when the decisions are not simultaneous, i.e. the male or the female decides before its mate, this has profound implications for the predicted pattern of care (Barta et al. 2002; McNamara et al. 2002). Whilst these arguments have firm theoretical foundations, the behavioural interactions that lead to desertion are rarely investigated in nature (but see Beissinger 1987; Valera et al. 1997).

We investigated parental interactions in a small passerine bird, the Penduline Tit, *Remiz pendulinus,* (body mass 9-10 g) that exhibits an unusually variable breeding system (Persson & Öhrström 1989; Szentirmai 2005). Unmated males start building sophisticated nests and sing to attract a female. Male and female finish the building of their nest together. Shortly thereafter, once the female has started egg-laying, either the male, or the female or both desert the nest during the egg-laying period. A single parent (the male or the female) always incubates the eggs and rears the young (Persson & Öhrström 1989; Valera et al. 1997). The main benefit of desertion is re-mating and reproducing with a new mate: deserting parents may remate up to five or six times within a single breeding season (Szentirmai et al. 2007). Approximately 30% of clutches are abandoned by both parents before incubation commences, presumably as a cost of the intense race for new mates (Persson & Öhrström 1989; Valera et al. 1997; Arnqvist & Rowe 2005). Biparentally deserted clutches are doomed to failure. By deserting the clutch, the parent offloads care to its mate, whereas he (or she) may increase his (or her) own reproductive success by abandoning the clutch, and mating with a new partner.

We had two objectives in this study. First, we measured whether desertion was simultaneous or sequential in biparentally deserted clutches. In taxa with internal fertilisation, such as birds, the male releases sperm before the female lays the eggs. Thus whilst the female forms the egg, the male can abandon her (Dawkins & Carlisle

1976), and this suggests that desertion first by the male might be expected. On the other hand, Valera et al. (1997) argued that female Penduline Tits can desert first, because they are able to manipulate the males by concealing the information on the progress of egg-laying by covering the eggs and expelling the male from the nest chamber. Second, if a parent prepares to desert first, he/she may reveal this intention. For instance, Beissinger (1987) noted that in Snail Kites *Rostrhamus sociabilis*, in which either the male or the female can desert during brood rearing, provisioning of nestlings was significantly lower in the deserting parent. Thus the would-be deserter appears to save energy by reducing its parental contribution. In this study, we tested whether either vocal or nest-building behaviour of parent Penduline Tits predicted later desertion.

**METHODS**

We studied the Penduline Tits between April and August in four consecutive years (2002-2005) in southern Hungary (46˚ 19' N, 20˚ 5' E) at an extensive fishpond (Fehértó, 1321 ha) where approximately 60-90 males and 45-50 females bred each year on the dykes separating fishpond units. The number of nests per year varied from a minimum of 158 in 2005 to a maximum of 214 in 2002. Penduline Tits were ringed with a unique combination of colour rings and a numbered metal ring (see Bleeker et al. 2005). Returning rates of individuals across years are very low: Out of 248 colour-ringed males, only 15 males were re-sighted in our area in a next year between 2002 and 2005. For females, out of 125 colour-ringed females, only 9 were seen in more than one year (van Dijk, RE, unpubl. data).

We visited nest-building males nearly every other day (1.8 ± 0.8 days [mean ± SD], $n$ = 59 nests), and watched them for at least 15 minutes and checked whether the male was mated and, for mated pairs, which of them attended the nest. Fifteen minutes were sufficient to record the presence of parents (if they were still at the nest), since males and females were spotted at the nest within 3min 41s ± 5min 11s (SD) and 6min 48s ± 7min 31s, respectively ($n$ = 46 nests where detailed behavioural observations were carried out). We considered a male to be mated when the pair copulated near the nest, or when the male and female built the nest together. A parent was considered to have deserted the clutch if it was not seen at the nest for at least two consecutive visits. None of the birds classified as 'deserted' returned to their nest afterwards. Desertion always took place during egg-laying and never before egg-laying or during incubation. Pair-bonds were short and lasted for 4.7 ± 2.6 days ($n$ =

315 nests). Nests with female-only care contained a clutch of 5.8 ± 1.3 eggs ($n$ = 140 nests), whereas nests cared for by males contained 3.5 ± 1.4 eggs ($n$ = 35 nests). 109 nests were deserted by both parents. The clutch size was known for 82 biparentally deserted nests; these had 3.0 ± 1.3 eggs. 19 biparentally deserted nests out of 109 were checked daily, whereas the remaining nests were checked approximately every other day. We tested whether one sex deserts before the other in biparentally deserted nests using a binomial distribution with $P$ = 0.5. In this test more than one nest of a given individual in a given breeding season may have been included (out of 98 colour-ringed males in the 109 biparentally deserted nests $n$ = 22 males; out of 29 colour-ringed females in the 109 biparentally deserted nests $n$ = 6 females). However, the constitution of pairs was always different.

We observed the behaviour of newly mated pairs in 2003, 2004 and 2005. The observations were carried out from a hide at a distance of 10-15m from the nest, using binoculars. We observed each pair for about two hours each day (124 ± 50 minutes, mean ± SD). Observations were alternated for pairs between morning (05:50 to –12:40 h, CET) and afternoon (11:30 to –19:50 h), thereby controlling for possible effects of time of day. Behavioural observations were collected until one or both of the parent(s) deserted the clutch. Every 20 seconds the following behaviours were recorded separately for the male and the female within a radius of 10m around the nest: nest building, calling, and, for males only, singing. The percentage of each behavioural score was calculated for each record assuming that these behaviours only take place around the nest. Twenty-five nests were observed: 6 were cared for by the male, 12 by the female, and 7 were deserted by both parents. At 19 nests both the male and the female were individually colour-ringed, whereas at six nests only the male was ringed. Of six unringed females, three bred at the same time, so we are certain that these were different individuals. For the remaining three females we cannot exclude the possibility that we observed the same individual more than once, although this is unlikely given the size of the study population. One observer collected all behavioural records at 24 nests, whereas one nest was studied by two observers. We found no significant effect of season (i.e. date of desertion), observer or year on the behaviour of male or female (MANOVA; all $P > 0.524$).

We used multinomial logistic regression models to predict parental care (male-only care, female-only care, biparental desertion) in response to the prior behaviour of the male (model (a) in Table 3.1) or the female (model (b) in Table 3.1) separately as covariates. Both the model for the male behaviour and the model for the

female behaviour predicting parental care fitted the data (Pearson's Goodness-of-Fit; male: $\chi^2$ = 45.476, df = 42, $P$ = 0.329; female: $\chi^2$ = 50.980, df = 44, $P$ = 0.218). Statistical analyses were performed using SPSS 11.5.0 and SPSS 12.0.0 for Windows.

## RESULTS

All nests included in this study were deserted by one or both of the parents during egg-laying. The pattern of parental care at nests in our population was similar to that reported by others (e.g. Franz & Theiss 1983; Persson & Öhrström 1989): about 11% had male-only care, 49%

**Table 3.1.** Multinomial logistic regression models of parental care strategy in response to (a) male behaviour and (b) female behaviour (n = 25 nests, df = 1). Separate models were constructed for (a) and (b). The reference category is biparental desertion. Predicted effect sizes and standard errors are given.

(a)

| Care strategy | | Model effect estimate (± SE) | Wald | *P* |
|---|---|---|---|---|
| Male-only care | Building | -0.162 ± 0.114 | 2.003 | 0.157 |
| | Calling | -0.309 ± 0.519 | 0.354 | 0.552 |
| | Singing | 0.113 ± 0.476 | 0.056 | 0.812 |
| Female-only care | Building | -0.097 ± 0.103 | 0.901 | 0.342 |
| | Calling | -0.399 ± 0.460 | 0.751 | 0.386 |
| | Singing | 0.553 ± 0.406 | 1.857 | 0.173 |

(b)

| Care strategy | | Model effect estimate (± SE) | Wald | *P* |
|---|---|---|---|---|
| Male-only care | Building | 0.046 ± 0.061 | 0.553 | 0.457 |
| | Calling | -0.589 ± 1.215 | 0.235 | 0.628 |
| Female-only care | Building | -0.049 ± 0.063 | 0.621 | 0.431 |
| | Calling | -1.582 ± 1.292 | 1.500 | 0.221 |

had female-only care, and 40% suffered desertion by both parents (Szentirmai 2005). Of the 19 biparentally deserted nests which we checked daily, 73.7% ($n$ = 14) were deserted by both parents on the same day, 10.5% ($n$ = 2) were deserted first by the male, and 15.8% ($n$ = 3) were deserted first by the female (Fig. 3.1). At those biparentally deserted clutches where one parent deserted first, the other parent

always deserted by the next day ($n$ = 5). These results are consistent with the total sample of biparentally deserted nests, including those that were checked approximately every other day: 78.9% ($n$ = 86), 11.0 % ($n$ = 12), and 10.1% ($n$ = 11), respectively (Fig. 3.1). Thus, the majority of biparentally deserted clutches are deserted by both parents on the same day. The frequencies of nests deserted first by the male and those deserted first by the female were not different from binomial distribution ($n$ = 23, $P$ = 1.000). When we took only one nest randomly per individually identified male and female, the pattern remains consistent with our previous results (both parents on the same day: 71.4% [n = 10], male first: 7.1% [n = 1], female first: 21.4% [n = 3]).

The behaviour of neither male nor female predicted which of the parents, male or female or both, would desert the clutch (Table 3.1).

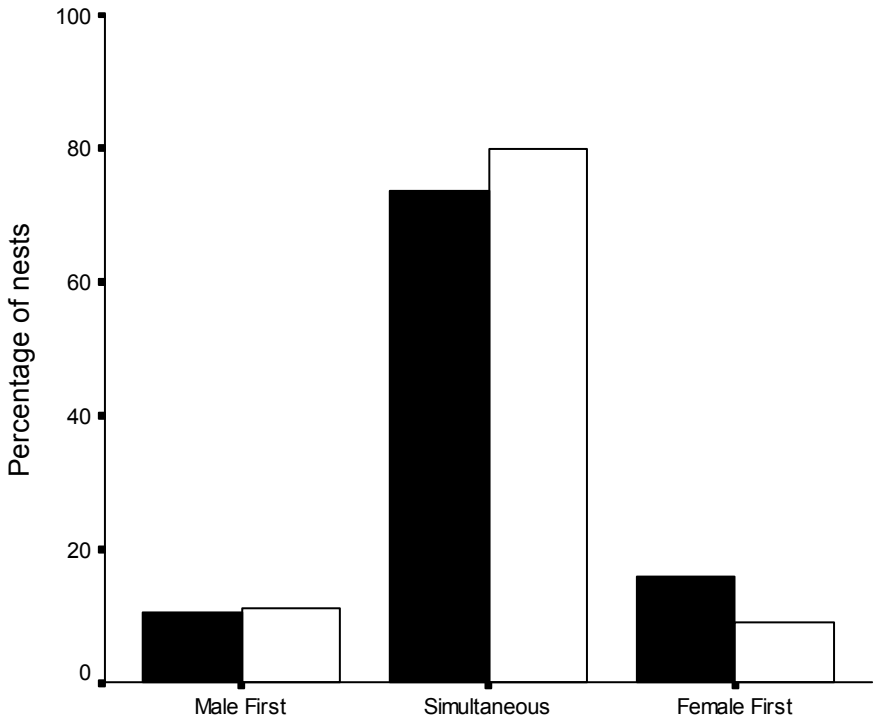



**Figure 3.1.** Percentage of Penduline Tit nests deserted by the male first, female first and by both parents simultaneously using nests that were checked daily (filled; $n$ = 19 nests), or less regularly (open; $n$ = 109 nests).

## DISCUSSION

Biparental desertion is a rapid process in the Penduline Tit, since most biparentally deserted nests were deserted by both parents on the same day. In our population of Penduline Tits, neither sex consistently initiated desertion. This result is different

from Persson and Öhrström's (1989) observation of a Swedish population which suggested that in the biparentally deserted nests it was always the female that deserted first, since they never observed a female at a nest after the male had deserted. Interestingly, Valera et al. (1997) reported a different pattern in an Austrian population of Penduline Tits (see their Table 1, page 24): males deserted earlier (1.6 ± 0.1 days after the start of egg-laying) than females (2.3 ± 0.1, Mann-Whitney U, $Z$ = ± 3.403, $P < 0.001$). Note that Valera et al.'s data appear to include both uniparentally and biparentally deserted nests, so they are not directly comparable to our data (see Fig. 3.1). Nevertheless, the comparison between these three detailed studies suggests that there are differences between populations in the process of desertion.

Although we found no consistent pattern within the Hungarian population, some individuals may consistently desert earlier than others. For instance, attractive males (i.e. males with a larger mask that have a significantly higher chance of attracting a female and need a shorter time to attract a female than males with smaller masks (Szentirmai 2005)) may desert their mate sooner than unattractive ones. Recently, Bleeker et al. (2005) showed that parent Penduline Tits in good body condition are more likely desert than those in poor condition. Thus parental care strategy (desert or care) is at least partially state-dependent.

One may expect an arms race for desertion between male and female Penduline Tits, since each parent may desert first as a pre-emptive strike, in order to oblige its mate to stay and care for the offspring. Such pre-emptive actions have been analysed in game-theoretic models (Lazarus 1990; Barta et al. 2002), although the relevance of these theoretical models in natural populations needs further scrutiny. Furthermore, desertion can be viewed as an interactive process in which the parents negotiate over care provisioning (McNamara et al. 2002). In such an arms race it may be a good strategy to disguise the intention to desert. Indeed, from our behavioural observations, it seems that Penduline Tit parents do not signal or reveal any intention to desert in their behaviour close to the nest.

In conclusion, we showed that clutch desertion is a rapid process in Penduline Tits, since the decision (care/desert) is made within a day, or at most within two days, at biparentally deserted nests. We need further studies to establish whether desertion strategies are consistent for a given individual, and to evaluate how state-dependency influences parental strategies. Future work with an increased resolution, *e.g.* using an interval recording camera to record the behaviour of parents at their

nest over full day, will be important to reveal the subtle interactions between males and females during the critical days of pair-formation and egg-laying.

**ACKNOWLEDGEMENTS**

We thank Gábor Bakacsi, Dušan Brinkhuizen, Rob Gordijn, Kenneth Hayes, Szabolcs Kajdocsi, Arno wa Kang'eri, Otília Menyhárt, Lídia Mészáros and Arjen Pilon for assistance in the field. Kiskunsági National Park and Szegedfish Kft. kindly permitted us to work and live in Fehértó, Hungary. Franjo Weissing provided us valuable statistical advice. We thank Michael Brooke, Jim Reynolds and one anonymous reviewer for comments on a previous version of this paper. Financial support was provided by Marco Polo Fonds and Groninger Universiteitsfonds to REvD, and grants to TS (Hungarian Scientific Foundation OTKA T031706, T043390, The Royal Society 15056 and the BBSRC BBS/B/05788). TS was also supported by The Leverhulme Trust (RF/2/RFG/2005/0279).

# CHAPTER IV

## EURASIAN PENDULINE TITS CONCEAL THEIR INTENTION TO DESERT THEIR MATE

**René E. van Dijk, Jan Komdeur & Tamás Székely**

*Manuscript*

*Authors' contributions*

**RevD**: study design, data collection, statistical analysis, manuscript preparation

**JK**: manuscript improvement

**TS**: study design, manuscript improvement

**SUMMARY**

Should parents advertise - or conceal - their intentions to provide care? Parents are expected to signal their abilities to provide good care for the young since this may make them more attractive and enhance the reproductive effort of their mate. In many animals, however, one parent abandons the young. This is costly to the mate, because the deserted parent then bears the full cost of care provisioning. In the latter situation signalling bad parenting unlikely evolves, because it would not benefit the would-be deserter. We tested these propositions in a small passerine bird, the Eurasian penduline tit *Remiz pendulinus* that has a highly variable breeding system: both males and females are sequentially polygamous (up to 6 mates in a single breeding season), and only one parent (either the male or the female) incubates the clutch and rears the chicks. Here we focus on the short time-window of egg-laying, during which desertion takes place. On the one hand, we predicted that the behaviour of would-be deserter changes nearer desertion, for instance he/she reduces the activities around the nest. On the other hand, deserters may disguise their intention to desert given that desertion is costly to their mate. We investigated the behaviour of 20 pairs of penduline tits in southern Hungary. By analysing 232816 video frames we show that nest attendance prior to desertion does not predict offspring desertion by either the male or the female. This result is consistent with the argument that would-be deserters should conceal their likely action in future. An alternative proximate-level explanation is that desertion is a rapid process perhaps triggered by internal or external stimuli. Penduline tits respond instantaneously to these stimuli, and this process is so fast that the mate has no time to exhibit counter-measures.

## INTRODUCTION

The behaviour of the partner is expected to play an important role in the decision how much to invest in a current brood (Houston & Davies 1985; McNamara et al. 2002; Houston et al. 2005; *Chapter II*). Additionally, given the costs of parental care in terms of time and energy (Clutton-Brock 1991), the interests of the individual male and female parent as for how much care to provide are often different ('sexual conflict over parental care'; Trivers 1972; Houston et al. 2005). Parental interactions during which parental care may be negotiated have been suggested to play an important role in resolving the conflict (Houston & McNamara 1999; McNamara et al. 1999; Johnstone & Hinde 2006). The outcome of these negotiations and the extent to which parents respond to each other appears to vary widely both across and within species, from complete insensitivity to the other's behaviour (e.g. Schwagmeyer et al. 2002; Steinegger & Taborsky 2007) to a clear response in a positive ('matched response'; Griggio et al. 2004; Hinde 2006) or negative direction ('compensatory response'; Houston & Davies 1985; Griggio & Pilastro 2007; Kosztolanyi et al. 2009). One possible explanation is the antipodal predictions as to whether parents should adjust their behaviour in relation to future parental investment (e.g. Kokko 1998; Osorno 1999; de Heij et al. 2006), or whether they would be better off disguising their decision over parental care. In snail kites (*Rostrhamus sociabilis*) deserting parents provisioned nestlings with less food prior to desertion, so as to save energy that may be invested in future reproduction, whilst at the same time the mate's abilities to care for the offspring may be tested (Beissinger 1987; Bart & Tornes 1989; Kokko 1998; Barta et al. 2002). Alternatively, a parent may benefit from concealing its intention to desert to prevent a pre-emptive strike of being deserted by its partner (Lazarus 1990; Barta et al. 2002; *Chapter III*). Because of the latter, also the timing of desertion – which parent should desert first? – will be important for the process of desertion. If there is a pattern for males and females to desert at a certain time of the day, this may have important implications on the process of desertion as the predictability of desertion increases. Nest desertion by males and females is likely associated with the progress of egg-laying (Dawkins & Carlisle 1976; Valera et al. 1997). Males may risk losing paternity if they desert too early. Females will be constrained by egg production, but deserting too late may result in desertion by their mate, whilst deserting too early may reduce the chance their mate will stay and care (Schleicher et al. 1997; Valera et al. 1997; Arnqvist & Rowe 2005). Despite extensive theoretical interest, an empirical understanding of

parental interactions in nature is only gradually accumulating (Beissinger 1987; Valera et al. 1997; Griggio et al. 2004; Hinde & Kilner 2007; Steinegger & Taborsky 2007; *Chapter III*). Here we investigate how parental interactions may predict nest desertion in sequentially polygamous Eurasian penduline tits.

Eurasian penduline tits exhibit a uniquely diverse mating and breeding system in which, likely as a result of sexual conflict, 80-95% of nests is deserted by the male, 30-70% by the female and about one third of nests is deserted by both parents (Szentirmai et al. 2007). After desertion, both males and females may remate with up to 6 mates in a given breeding season: sequential polygamy by both sexes commonly occurs. In an earlier study, using every other day nest checks and two-hour behavioural observations at a daily basis, we suggested that male and female penduline tits desert simultaneously, and that neither parent behaviourally signalled its intention to desert (*Chapter III*). Yet, the association of these behaviours with parental care decisions may be subtle. A parent may benefit from preventing an increase or reduction in parental contribution to become sufficiently high to convey information about the intention to desert, to avoid being deserted as a pre-emptive strike by the partner (Lazarus 1990). The aim of this study was to pick up such subtle behavioural changes using uniquely detailed behavioural video observations at the nest.

Firstly, we investigated if nest attendance could predict the decision about parental care. We predicted that the deserting parent will attend the nest less frequently than the parent that is going to care for the offspring (see *Chapter III*). Our study is the first investigating nest desertion strategies using detailed behavioural video recordings.

Secondly, we determined which parent deserted first at biparentally deserted nests. Desertion by one of the parents likely has a profound influence on the predicted decision about parental care by the parent that is left behind (Barta et al. 2002; McNamara et al. 2002; *Chapter III*).

Finally, we determined at what time of the day male or female parents desert their nest. In our population, 36.5% of nests contained at least one extra-pair young (Mészáros, AL, van Dijk, RE, van der Velde, M, Komdeur, J, Székely, T, & Szabad, J unpubl. data). Given the apparent risk for males to lose paternity, we expect males to mate guard their females and only desert just after the female has laid an egg to ensure paternity (Birkhead & Møller 1992; Schleicher et al. 1997; Komdeur et al. 2007; Johnsen et al. 2008). Females may trade-off the risk of deserting too early with

a reduced chance of their mate staying behind to care, with the risk of deserting too late, resulting in being deserted by their mate, but we expect they will desert as soon as an egg has been produced to reduce the latter risk. We thus expect both males and females to desert in the early morning.

## 2. MATERIAL AND METHODS

*Study site and data collection*

Fieldwork was conducted in a 1,321 ha reed-marsh, Fehértó, in southern Hungary (46º19'N 20º6'E) between April and August in 2006 and 2007. If a parent had not been observed at the nest for at least 15min at two consecutive nest checks ($n$ = 14 individuals; 10 males and 4 females; see below) or for at least one hour using our detailed video observations ($n$ = 14 individuals; 5 males and 9 females; see below), it was classified as 'deserted'. Once classified as such, none of these individuals were observed at the nest again (see Bleeker et al. 2005 and *Chapter III* for detailed field methods).

We investigated nest attendance by male and female Eurasian penduline tit using video recordings during daylight hours during the period between pair formation and nest desertion (2.50 ± 1.36 days of video recordings per pair, 302min ± 170min per day, $n$ = 20 pairs). During this period both parents build the nest after the male has initiated the nest before pair formation (Hoi et al. 1994). Using a time-lapse video camera (Sony DCR-HC44E), set up at approximately 10m from the nest using up to 12x optical zoom, we stored one picture every five seconds. A total sample of 232816 frames was analysed frame by frame using MATLAB v. 6.5, coding presence on or inside the nest as: (i) male-only, (ii) female-only, (iii) simultaneous nest attendance by male and female, or (iv) both parents absent. All recordings were analysed by one person only (REvD). We only included pre-desertion records.

To distinguish male and female parents from intruders we used colour rings, individual differences in plumage (Cramp et al. 1993; Kingma et al. 2008) and behaviour (e.g. the presence/absence bouts of females are longer than for males; intruders are often on the outside of the nest and build very little). At 39.3% of all frames recorded we were able to read the colour ring combination. In addition, when the birds are inside the nest the head is frequently visible. This makes it possible to distinguish the sexes based on eye-stripe size and crown feather colouration (Kingma et al. 2008; *Chapter IX*). In those cases we assumed that this was the same individual

for which we read the colour ring combination at the arrival of the bird at the nest. This is realistic given the high resolution of our recordings (one photo every fifth second) and the length of the presence/absence bouts (see Results). Frames where the identity of the bird was ambiguous were omitted from the analyses (7.4% of total of recorded frames). Out of the 20 pairs filmed, three males and 14 females were not colour ringed. However, five out of seven unringed females in 2006, three unringed out of seven unringed females in 2007, and both unringed males in 2007 bred simultaneously, and we can thus be certain these are different individuals. We unlikely observed the same individual in two years as adult returning rates between years are low (*Chapter VII*).

*Data analyses*

We calculated, for males and females separately, the percentage of time a parent was present at the nest as well as the number of nest visits per hour ('nest attendance frequency'). For the latter we excluded data from recordings that were made for < 60min per hour. For time in season we used a date format as the number of days since 1 March in each year.

We used binary and multinomial logistic regression models (Hosmer-Lemeshow; $p > 0.141$; multinomial logistic regression with biparental desertion as the reference category: Pearson goodness-of-fit; $p > 0.297$) to predict parental care in response to male or female nest attendance. We used linear mixed models with restricted maximum likelihood (LMM) to test whether (i) the time of the day, (ii) parental care strategy, (iii) the time before desertion, and (iv) the interaction of the time before desertion and the parental care strategy predicts nest attendance (up to 116 hours before desertion). The covariates 'time of the day' and 'time before desertion' were used in all analyses at a two hour resolution. We also calculated the residuals for male and female total nest attendance per nest site. We therefore determined the function that provided the best fit over the time before desertion for each day separately and calculated the residuals using CurveExpert v. 1.37 with all model families selected and three degrees of polynomial to consider. The time of the day, parental care strategy, and the time before desertion, and their interaction were entered as fixed effects, and nest site as subject grouping with the time before desertion and the time of the day as repeated variables. We applied correlation metric compound symmetry as the repeated covariance type. The assumption of normally distributed data was violated for the models presented, but this was caused by

outliers and we thus anticipate that this unlikely will have had a considerable influence on our models' results (Sokal & Rohlf 1995). We used separate models for total nest attendance and nest attendance frequency, as well as for the sexes.

To test for a pattern in the timing of desertion, we bootstrapped the timing of males and females, and compared these with random timing of desertion for males and females separately. These random timings were extracted 1000 times from a uniform distribution with the minimum and maximum timing of desertion given by the observed minimum and maximum for males and females. All statistical analyses were performed using SPSS 14.0.0 (SPSS Inc., USA).

**Table 4.1** Results of the binary logistic regression models of male and female care strategy in response to (a) total nest attendance by male and female separately, i.e. male- and female-only plus simultaneous nest attendance ($n$ = 20 pairs, df = 1) and (b) male and female nest attendance frequency per two hours ($n$ = 19 pairs, df = 1). Separate models were constructed for (a) and (b). Predicted effect sizes and standard errors are given.

(a)

| | Total attendance | Model effect estimate (± SE) | Wald | *P* |
|---|---|---|---|---|
| Male behaviour | Male | -0.055 ± 0.075 | 0.526 | 0.468 |
| (care versus desert) | Female | 0.027 ± 0.087 | 0.094 | 0.759 |
| Female behaviour | Male | -0.090 ± 0.075 | 1.442 | 0.230 |
| (care versus desert) | Female | 0.007 ± 0.082 | 0.007 | 0.934 |

(b)

| Care strategy | Frequency | Model effect estimate (± SE) | Wald | *P* |
|---|---|---|---|---|
| Male care versus | Male | 0.022 ± 0.099 | 0.048 | 0.826 |
| male desertion | Female | -0.250 ± 0.218 | 1.314 | 0.252 |
| Female care versus | Male | 0.203 ± 0.132 | 2.346 | 0.126 |
| female desertion | Female | -0.143 ± 0.195 | 0.537 | 0.464 |

## 3. RESULTS

*Does nest attendance predict parental care?*

Five (25%) nests out of the 20 were deserted by the female, seven nests (35%) by the male and eight nests (40%) were deserted by both parents.

We found no effect of year or date of nest desertion on total nest attendance by the male or female ($F > 0.005$, $p > 0.256$, $n = 20$). Males and females attended the nest in bouts of 1min14s ± 1min53s and 3min46s ± 4min29s, respectively, whereas

they were absent from the nest for on average 5min13s ± 6min50s and 12min36s ± 14min46s ($n$ = 20 males and 20 females). Of all recorded periods of male and female absence, 93.0% and 69.4% was shorter than 15min, respectively, and 98.5% and 90.0% was shorter than 30min. This suggests that using 15min observations during nest checks was usually enough to score the presence of male and females at the nest and that two observations were enough to determine if a parent had deserted (see Methods).

Neither mean total nest attendance by male or female, i.e. male- or female-only plus joint nest attendance (Table 4.1a), nor nest attendance frequency by male or female (Table 4.1b) predicted whether the male or female parent deserted the clutch.

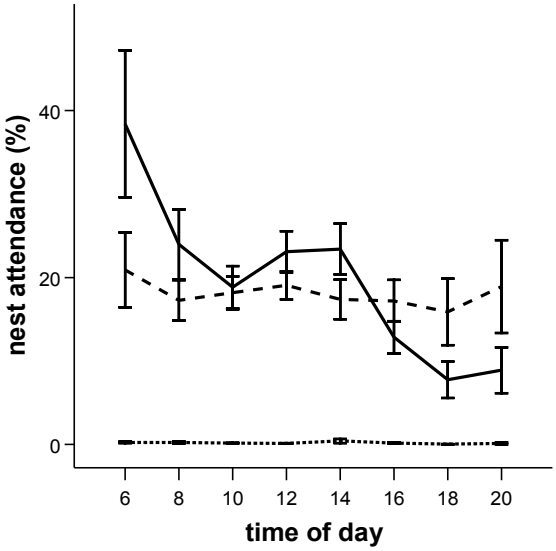



**Figure 4.1** Male-only, female-only, and simultaneous nest attendance over the time of the day (mean ± SE). Time period at two hour resolution (for instance, 6 = mean attendance from 4am until 6am). $n$ = 20 pairs
continuous line = female-only; striped line = male-only; dotted line = simultaneous nest attendance

We found that males reduced their total nest attendance (LMM; $F$ = 9.253, $p$ = 0.003) and nest visit frequency ($F$ = 8.816, $p$ = 0.004, $n$ = 20 males) over the period before desertion, whereas females only reduced their total nest attendance ($F$ = 37.725, $p$ < 0.001) and nest visit frequency ($F$ = 6.913, $p$ =0.010, $n$ = 20 females) over the time of the day (Fig. 4.1). However, when we included only observations of up to 48 hours before desertion took place, thereby increasing the number of nests per time unit from 2.37 nests to 5.21, the above effect for male nest attendance disappeared ($p$ > 0.312, $n$ = 20; Fig. 4.2a), whereas the effect of the time of day on female nest attendance remained significant ($F$ = 25.399, $p$ < 0.001 for total attendance, and $F$ = 4.703, $p$ = 0.034 for nest visit frequency; $n$ = 20; Fig. 4.1). Neither parental care strategy, nor the interaction with the period before desertion predicted male or female total nest attendance or nest visit frequency ($p$ > 0.126; Fig.

4.2). Similar results were found using the residuals per day from the curve providing the best fit: Female nest attendance was significantly associated with the time of day for the analysis including all days, as well as for the last 48 hours only (LMM; $p < 0.001$, $n = 20$ nests; Fig. 4.1). All other effects were non-significant ($p > 0.052$).

*Timing of nest desertion*

Desertion was a rapid process. Out of eight biparentally deserted nests, at five we knew when both parents deserted. These parents deserted within little more than 1day after each other: one nest was deserted first by the female, after which the male deserted 2h21min45s later. The other two nests were deserted by the male first, after which the female deserted at one nest after 1day and 21min5s, and at the other nest the female deserted only 25s after the male. At a further two nests, one female deserted at 15:36:45, after which the male deserted the nest between 19:37:30 the same day when we left the nest and 5:35:00 the next morning when we came back. At the second nest the female deserted at 13:58:40, and the male deserted the nest later as well at an unknown time. At the remaining nests we were unable to determine the sequence of desertion.

The exact timing of desertion has been established for 5 males and 9 females at 20 nests. Females deserted throughout the day (Fig. 4.3). Timing of desertion by females was not different from the random timing of desertion ($Z = 1.592$, $p = 0.111$, $n = 2000$). In contrast, males tended to desert either in the morning or late afternoon/evening (Fig. 4.3): two males deserted before 7.00am, three after 4.00pm ($Z = 4.808$, $p < 0.001$, $n = 2000$). An additional five males deserted between 17:18:25 and 7:45:00 (Fig. 4.3), but the exact timing of these males was unknown.

## 4. DISCUSSION

Consistent with our previous study (*Chapter III*), we show that i. the behaviour at the nest by either sex does not predict which parent will desert, ii. neither sex consistently deserted first and iii. females may desert throughout the day, whereas males deserted either in the early morning or late afternoon. To our knowledge this is the first study pointing out a sex difference in timing of desertion at a daily resolution.

**Figure 4.2** The pattern of nest attendance over the period before desertion for three parental care strategies. Mean residuals of nest attendance from best fit curve per day at a two hour resolution; mean ± SE. For each day, as separated by the grey dotted line, a separate function was fitted. Desertion takes place at x = 0. (a) Mean residuals of nest attendance by the male, (b) mean residuals of nest attendance by the female. Nests were not filmed during the night or during bad weather, hence the gaps. *N* = 20 pairs in total.

continuous line = female-only care; striped line = male-only care; dotted line = biparentally desertion

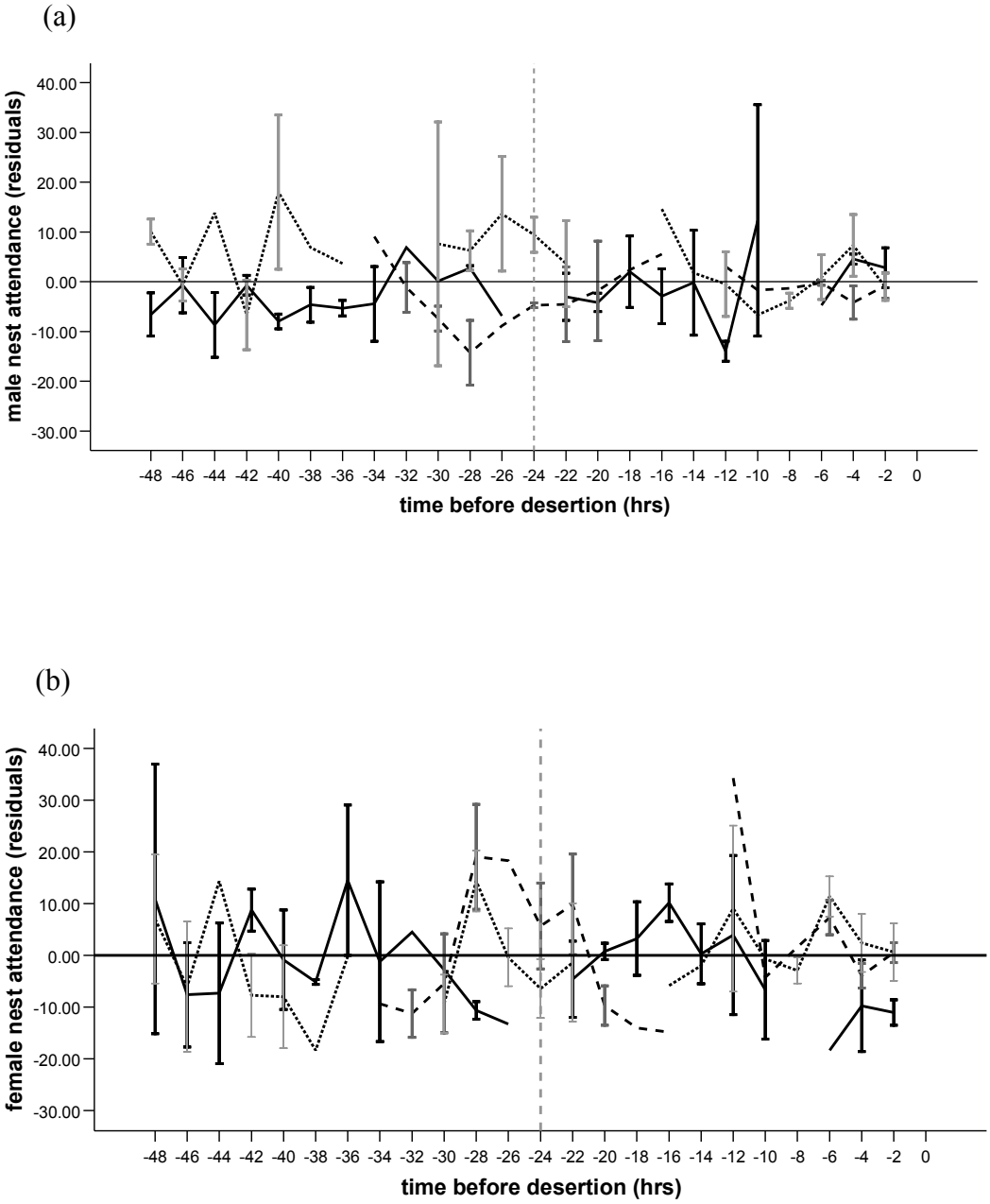
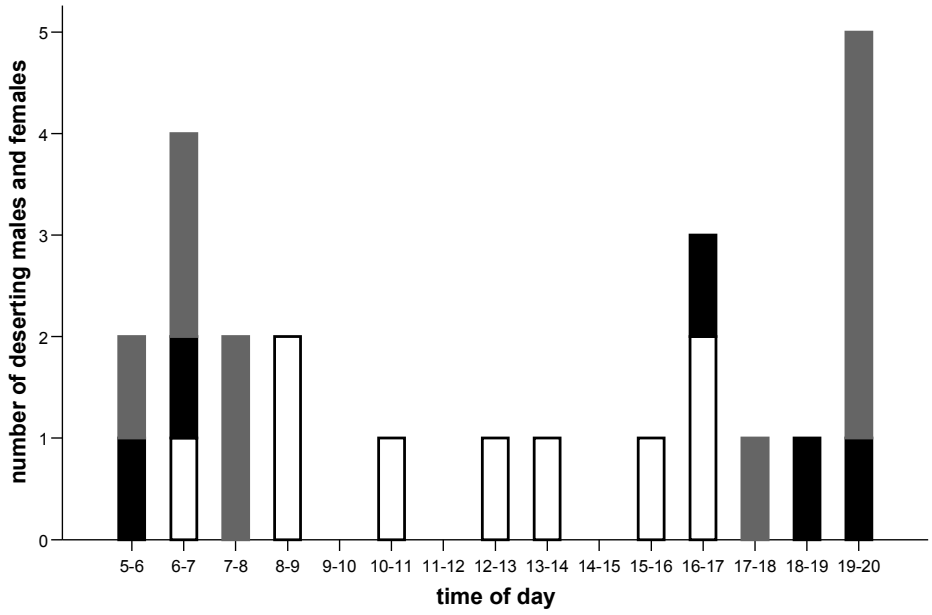


**Figure 4.3** Timing of desertion by males (black) and females (white); shaded boxes indicate those males that deserted either in the evening or early morning




*Behavioural cues for nest desertion*

Although there appears to be no strict 'cruel bind' given that some nests are sequentially deserted, the behaviour of one of the parents is likely influenced by that of its partner (McNamara et al. 2002). In this paper, using more detailed behavioural observations, we found support for our argument that Eurasian penduline tits may be better off concealing their decision about parental care to pre-empt being deserted by their mate (*Chapter III*): male and female nest attendance prior desertion could not predict the parental care strategy adopted. Parents do not appear to adjust their behaviour as the time that one (or both) of the parents is going to desert is approaching, nor do they adjust their behaviour to the parental care strategy adopted at a given nest.

Alternatively, our results on the timing and sequence of desertion suggest that the decision to care or desert is only made within a small time window around the third day of egg laying, i.e. just before they actually desert (see below). However, it seems likely that potentially confounding variables influencing the decision to care or desert, such as the availability of mates, their attractiveness, and food availability, are being assessed over a more extended period during and perhaps before the egg laying phase.

The daily pattern found for females may be confounded by a multitude of variables, but one possible explanation is the potential for success of extra-pair copulations: Because females are least receptive around egg laying (Birkhead et al. 1996), the best time for females to search for and solicit extra-pair copulations should be during the afternoon and early evening. If she is to invest in nest building, the best time for that in this respect would indeed be during the early morning.

*Timing of desertion*

The fact that at biparentally deserted nests, neither the male, nor female consistently initiated desertion and that the parents deserted either on the same day (with one nest where the interval between desertion of both parents was as little as 25sec), or at most within two days supports the suggestion that the process of desertion is rapid (*Chapter III*). It also suggests that desertion as a pre-emptive strike to avoid to be deserted is not always a successful strategy in penduline tits: both males and females may escape the 'cruel bind' (Dawkins & Carlisle 1976; Lazarus 1990; McNamara et al. 2002; Griggio et al. 2004).

Certainty over paternity might drive males to desert either in the late afternoon/early evening or in the early morning, but not late morning/early afternoon. The high population density in our study area and the polygamous nature of the penduline tit's breeding system suggest that sperm competition may be intense. For males, guarding his mate until the evening or until the egg is actually laid the next morning so as to ensure his sperm will fertilise the egg may thus be a worthwhile strategy. However, mate guarding may not be intense, since the parents spend very little time together at the nest (Fig. 4.2; see: Schleicher et al. 1997). Deserting in the morning also allows time to forage and seek new mates, with a reduced risk of loosing paternity over future offspring, if the time around egg-laying is the period when insemination is least likely to result in fertilisation (Birkhead et al. 1996). The condition of males may confound why some males desert during the evening, whereas others desert in the morning: If a male is in a good condition, he may afford to spend the night outside the nest, whereas a male in a poor condition may need to spend the night inside the nest and benefit from its insulative capacities (Glutz von Blotzheim 1993; Schönfeld 1994; Szentirmai et al. 2005b). Our sample size currently does not allow us to distinguish which individual male traits may be related to timing of desertion.

Females are likely constrained by a minimum number of eggs they lay before it pays to desert (Persson & Öhrström 1989; Franz 1991; *Appendix I*), after which an assessment of other factors, such as progress of nest-building, food availability, or availability of mates, may determine when they will desert, which may show daily variation. Activity of males (female mating opportunities) appears not to change substantially over the course of the day (see Fig. 4.2), and also a dawn chorus, as exhibited by many songbirds, is typically absent in penduline tits (van Dijk, RE, Bot, S, & Pogány, Á, pers. obs.). This suggests that a sex difference in hormones is unlikely a proximate explanation behind the sex difference in timing of desertion.

Although this clearly requires further research, the sex difference in timing of desertion has important implications on the process of desertion: males seem somehow constrained as to when they may desert, whereas females may desert at any time during the day.

*Future avenues*

Further work on behavioural interaction between parents at very fine resolution is required to unravel how subtle adjustments of behaviour of animals may influence a resolution to sexual conflict over care. Although in our previous study (*Chapter III*) we pointed out that vocal behaviour could not predict parental care strategies, this could be a candidate trait that may be used to advertise the parental care decision for instance only just before desertion takes place. Parents of the Galilee St Peter's fish *Sarotherodon galilaeus*, appear to monitor each other's behaviour, and possibly negotiate over parental care, by leaving the eggs on the ground after fertilisation before either the male, the female or both pick them up for mouth incubation (Schwanck & Rana 1991; Balshine-Earn & Earn 1997). Experimental manipulation of parental behaviour prior to desertion, such as mate removal, likely further reveals new insights as to how parents decide over parental care.

**Acknowledgements**

We are grateful to all people who helped with the fieldwork in Hungary. We thank the Kiskunság National Park (23864-2-3/2006, 23864-3-2/2007) and Szegedfish Ltd. for permission to carry out fieldwork. We also thank Johan van der Dennen for writing the MATLAB script to analyse the time-lapse video recordings. The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002-2006) under contract n. 28696. The work was further supported by a University of Bath studentship to REvD; the Hungarian National Science

Foundation OTKA (T043390 to TS); a Royal Society Joint Project grant (15056 to TS); and the Leverhulme Trust (RF/2/RFG/2005/0279 to TS).

# CHAPTER V

# What games do penduline tits play?

**René E. van Dijk, Tamás Székely, Jan Komdeur & Franz J. Weissing**

*Manuscript*

*Authors' contributions*

**RevD**: data collection, statistical analyses, game-theoretic analyses, manuscript preparation

**TS**: manuscript improvement

**JK**: manuscript improvement

**FJW**: study design, game theoretic analyses, manuscript improvement

**ABSTRACT**

The Eurasian penduline tit, *Remiz pendulinus*, exhibits an unusually diverse breeding system where female-only care (50-70% of nests), male-only care (5-20%) and biparental desertion (30-40%) all occur within a single population. By means of a game theoretical approach, we investigate whether, and to what extent, this diversity can be understood in terms of evolutionarily stable strategies (ESSs). We model parental decisions as a single-shot two-person game where both players have two pure strategies ('care' and 'desert') and use field data to quantify the fitness consequences of caring and desertion for males and females separately. The payoff matrix resulting from the fitness estimates suggests that penduline tits are involved in a coordination game with two alternative ESSs: female-only care and male-only care. Why did neither of these ESSs spread and fixate in the population? To answer this question, we argue that the average payoff matrix of the population gives a poor representation of the conflicts between individuals. Using bootstrapping, we replace the population payoff matrix by a variety of payoff matrices for individual conflicts. Some of these correspond to a Prisoner's Dilemma (with biparental desertion as the only ESS), others have female-only care or male-only care as a unique ESS, and still others correspond to the coordination game with two alternative ESSs. We conclude that the predicted distribution of care corresponds reasonably well to the parental care observed in penduline tits.

## INTRODUCTION

Decisions about parental care are among the most important decisions for the life history of individual animals. In vertebrates, a diversity of parental care systems exists, ranging from cooperative breeding with biparental care and helpers, such as in meerkats, *Suricata suricatta*, (Manser et al. 2008) or in long-tailed tits, *Aegithalos caudatus*, (Sharp et al. 2008) to very limited parental care, such as in precocial birds or brood parasites (Clutton-Brock 1991; Reynolds et al. 2002; Langmore & Kilner 2007; McGraw et al. 2009; *Chapter II*). Of particular interest are species with variable parental care decisions, such as those which exhibit a combination of male-only, female-only and biparental care, for instance snail kite, *Rostrhamus sociabilis*, Coqui Antillano, *Eleutherodactylus johnstonei*, Kentish plover, *Charadrius alexandrinus*, St Peter's fish, *Sarotherodon galilaeus*, and Eurasian penduline tit, *Remiz pendulinus*, (Beissinger & Snyder 1987; Székely & Lessells 1993; Balshine-Earn 1997; Bourne 1998; Szentirmai et al. 2007). These species provide an excellent opportunity to test predictions of game-theoretic approach.

An intriguing example is the Eurasian penduline tit. Both sequential polygyny and sequential polyandry commonly occur in a breeding season, so that several combinations of parental care occur simultaneously within a single population. All incubation and feeding of nestlings is carried out by one parent, at 5-20% of nests by the males and at 50-70% by the females. Additionally, about one third of clutches is deserted by both parents (Persson & Öhrström 1989; *Chapter VIII*; Table 5.1). What may be the explanation of such a diverse breeding system?

Parental care is influenced by the social and non-social environment and often has major impacts on the reproductive output in many animals (Clutton-Brock 1991; Burley & Johnson 2002; Owens 2002; Székely et al. 2006; Charpentier et al. 2008; McGraw et al. 2009; *Chapter II*). A decision about parental care is mediated by a trade-off between the effort invested in a current brood versus the costs in terms of future survival and reproduction and by a trade-off between the quality and quantity of the offspring (Clutton-Brock 1991; Houston et al. 2005; *Chapter II*). Since care is costly and parents may breed several times in their lifetime with different individuals, there is a conflict between the parents whereby each tries to shunt care provisioning to its mate (Parker 1979; Houston et al. 2005; McGraw et al. 2009; *Chapter II*). The cost and benefits of care, and the behavioural interaction between the parents are all important predicting how the parents resolve their conflicting interests (McNamara et al. 2002; Houston et al. 2005; *Chapter II*).

**Table 5.1:** Parental care strategies in various populations of Eurasian penduline tits (means ± CI; after *Chapter VIII*) and those predicted by the bootstrapped observed payoffs with the coordination game solved using payoff and risk dominance (see Table 5.4; FC = female-only care, MC = male-only care, BD = biparental desertion). The patterns of parental care are not significantly different across the five European populations (*Chapter VIII*). The predicted pattern of parental care applying payoff or risk dominance of the bootstrapped individual payoffs is significantly different from the observed pattern in our population in Hungary.

| Population | FC (%) | MC (%) | BD (%) | *n* |
|---|---|---|---|---|
| Hungary (*Chapter VIII*) | 45.0 ± 0.2 | 16.7 ± 0.2 | 38.3 ± 0.2 | 60 |
| The Netherlands (*Chapter VIII*) | 50.0 ± 0.4 | 6.3 ± 0.3 | 43.8 ± 0.4 | 16 |
| Sweden (Persson & Öhrström 1989) | 47.9 ± 0.2 | 17.9 ± 0.1 | 34.3 ± 0.2 | 140 |
| Germany (Franz 1991) | 65.2 ± 0.2 | 6.7 ± 0.1 | 28.1 ± 0.2 | 89 |
| Austria (Franz 1991) | 54.2 ± 0.2 | 14.0 ± 0.1 | 31.8 ± 0.2 | 107 |
| **Range** | **45.0 – 65.2** | **6.3 – 17.9** | **28.1 – 43.8** | **412** |
| Payoff dominance ($\chi^2 = 71.82$, $P < 0.001$, $n = 534$ pairs) | 45.4 | 28.0 | 26.6 | 5000† |
| Risk dominance ($\chi^2 = 371.36$, $P < 0.001$, $n = 534$ pairs) | 48.5 | 38.5 | 12.9 | 5000† |

†Values based on bootstrapped payoffs

Since the seminal study by Maynard Smith (1977), parental conflicts are often analyzed by means of a game-theoretic approach. Evolutionary game theory allows deriving of predictions in situations where the fitness of an individual does not only depend on this individual's own behaviour, but also on the behaviour of other individuals in the population. Parental decisions are prototype examples for such frequency dependence, since the fitness ('payoff') of a parent who decided to care for the clutch may be quite different if the other parent also cares as opposed to desert the clutch.

**Table 5.2.** The breeding biology of Eurasian penduline tits: number of eggs and nestlings produced in the first nest of the breeding season, the probability to have at least one more successful breeding attempt after the first successful nest ('successful' meaning pair formation and egg laying took place), the number of successful subsequent breeding attempts and the number of eggs and nestlings produced in subsequent nests (means ± SD). *n* is the number of males or females.

| | | **male-only care** | | **female-only care** | | **biparental desertion** | |
|---|---|---|---|---|---|---|---|
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| first | eggs | 3.50 ± 1.13 | 4.00 ± 1.00 | 6.39 ± 1.15 | 6.15 ± 1.25 | 3.13 ± 1.14 | 2.31 ± 0.79 |
| | nestlings | 2.59 ± 1.57 | 2.07 ± 2.00 | 3.40 ± 1.97 | 3.37 ± 1.98 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| subsequent | probability successful | 0.06 | 0.67 | 0.74 | 0.38 | 0.34 | 0.83 |
| | number | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.88 ± 1.14 | 1.48 ± 0.77 | 1.77 ± 1.06 | 1.38 ± 0.52 |
| | eggs | 0.06 ± 0.24 | 2.45 ± 2.12 | 6.34 ± 6.05 | 2.61 ± 3.97 | 4.49 ± 4.66 | 7.12 ± 5.00 |
| | nestlings | 0.00 ± 0.00 | 0.74 ± 1.28 | 1.96 ± 2.52 | 0.95 ± 1.70 | 1.54 ± 2.54 | 3.52 ± 2.92 |
| | *n* | 18 | 3 | 46 | 52 | 47 | 6 |

The variable parental care in penduline tits is likely a result of sexual conflict, since after desertion, both males and females may remate with up to six mates within a given breeding season, and both parents appear to produce more offspring in a breeding season when they desert rather than care for the offspring (Szentirmai et al. 2007; Table 5.2), although the reproductive consequences of biparental care are not known, because this has not been observed in our population (*Chapter VIII*). Nest desertion takes place during egg-laying. Once the male has deserted, females often lay a few more eggs, so that male-only cared clutches and biparentally deserted clutches are significantly smaller than those cared for by females (Persson & Öhrström 1989; Valera et al. 1997; Szentirmai et al. 2007; *Chapter VIII*; Table 5.2). In addition, in biparentally deserted nests all offspring die (Persson & Öhrström 1989; Szentirmai et al. 2007). Hence, although offspring survival does not differ significantly between male-only and female-only cared clutches (*Chapter VI*), the caring/deserting behaviour has a major influence on both parents' reproductive output (Szentirmai et al. 2007; this paper).

Existing game models for parental care have proved to be a useful mathematical approach to understand how individuals interact and which strategic decisions they may adopt to resolve their conflicting interests (McNamara et al. 1999; Johnstone & Hinde 2006; McNamara & Weissing 2009). Game theory attempts to find the optimal strategy for an individual, which depends on the strategy adopted by other players in the game. It can help us to predict which strategy is resistant against any possible mutant strategy given the fitness payoffs for each strategy, i.e. the Evolutionarily Stable Strategy (ESS; Maynard Smith & Price 1973). Frequency-dependence of strategies likely has a profound influence on which strategy results in the highest fitness payoff, in particular for strategies in a social context, which includes parental care (McNamara & Weissing 2009). A game may involve a single interaction between the individuals where the history of previous interactions generally does not exist, or is not taken into account (Maynard Smith 1977; Webb et al. 1999), repeated interactions resulting in a continuous adjustment of strategies with evolutionarily stable levels of effort depending on the strategy of the opponent or previous interactions and the reputation of players (such as tit-for-tat; Axelrod & Hamilton 1981; Houston & Davies 1985; Milinski 1987), or repeated interactions based upon bargaining, where the outcome is negotiated leading to evolutionarily stable negotiation rules (McNamara et al. 1999). Whilst these game theoretic models in a parental care context elicited much empirical interest (Wolf et

al. 1991; Öst et al. 2007; Harrison et al. submitted), most of them have not been applied to a specific situation in the field or in captivity (with the exception of Balshine-Earn & Earn 1997). Our objective here is to develop game-theoretic models for the parental care patterns of penduline tits and investigate which model gives the best predictions in regards to the observed behaviour in nature.

## FITNESS ESTIMATES

*Study Site and Data Collection*

We studied Eurasian penduline tits during the breeding seasons between April and August in six consecutive years (2002 – 2007) at a 1,321 ha fishpond system, Fehértó, in southern Hungary (46º19'N 20º6'E), where approximately 60-90 males and 45-50 females are known to breed each year (Szentirmai et al. 2007; these are the number of individual we ringed, biased towards males given that females are more difficult to trap than males). We searched the study area for nest-building penduline tits, and visited each nest about every other day to determine the date of nest initiation, date of pair formation, which parent attended the nest, the date of desertion, number of eggs (at approximately the eighth day after commencement of incubation), and the number of nestlings (at ten days after hatching of the first egg; the number of nestling at the tenth day after hatching is highly correlated to the number of fledglings; Kingma et al. 2008; *Chapter III*). We trapped and ringed birds with one metal ring from the Hungarian Ornithological Institute and a unique combination of three color rings (A.C. Hughes, Middlesex, UK; see details in Bleeker et al. 2005). Males were usually trapped before incubation using mist nets, whereas females were usually trapped during incubation at the nest. Adult returning rates between years are low (5% for males, 2% for females; *Chapter VII*). Pseudoreplication was avoided by selecting only one datum per colour-ringed individual. Additionally, the composition of pairs was nearly always different between subsequent clutches: out of 194 colour-ringed pairs that produced a clutch between 2002 and 2007, only six pairs (3.1%) did not change mate at successive nests.

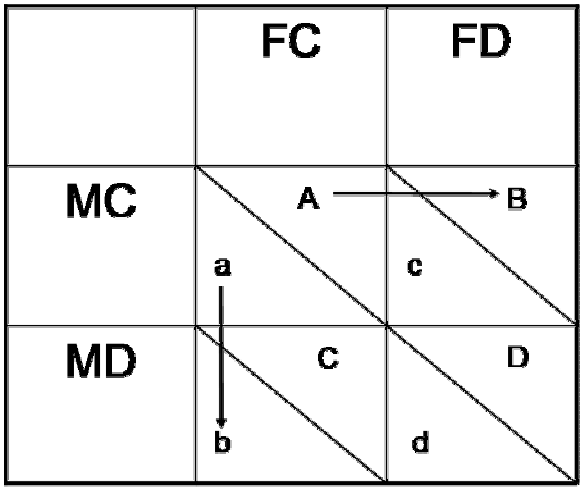
The pattern of parental care is consistent between populations of penduline tits across Europe and it does not appear to be associated with breeding density (Table 5.1; *Chapter VIII*).

*Estimating the Payoff Matrix*

We used field data to calculate the seasonal reproductive payoff for an individual, given the parental care strategy adopted at the first nest in a breeding season. Although the estimated number of fledglings for females is correct, for males the actual number may be influenced by the occurrence of extra-pair young. However, our pilot data suggest that although 36.5% of nests contains at least one extra-pair offspring, the number of extra-pair young do not appear to be associated with the parental care strategy adopted at a given nest (Mészáros, LA, van Dijk, RE, van der Velde, M, Komdeur, J, Székely, T & Szabad, J, unpubl. data). We therefore anticipate that it is unlikely that the existence of extra-pair paternity confounds our results. We focus on the strategy adopted at the first nest in the breeding season, since this likely has a major implication on the additional reproductive output that can be obtained at later nests in the same season (the incubation and nestling feeding period is approximately 34 days; Cramp et al. 1993; van Dijk, RE and Székely, T unpubl. data; Table 5.2). To define what the first nest in a breeding season was for a given individual, we took the nest initiation date of the first nest of known, banded males in our population in each year ($n$ = 267 males). The mean of these nest initiation dates was 3 June (SD = 22.7). We did the same for all second nests of males after a first successful nest, i.e. a nest were the male had attracted a female and a clutch was produced ($n$ = 101 males). The mean of the nest initiation dates of second nests was 8 June (SD = 21.1). We therefore included all nests of a banded individual male or female from the start of the breeding season until 6 June as first nests in a season in each year. The small difference between the mean nest initiation date of the first and second breeding attempts is due to males arriving throughout the season in our population. Some of these late arriving or early leaving males and females may have bred outside our population (Franz et al. 1987). We currently have no data to estimate the frequency of immigration, although the distance between subsequent breeding attempts is typically limited (Mészáros et al. 2006). This may, however, underestimate the seasonal reproductive success per individual, and may thus confound some of the variation predicted by our model. To calculate the seasonal reproductive success of individual males and females, we summed up for each individual the reproductive output (eggs or nestlings) in the first nest with that in all subsequent nests in a given breeding season. This was done separately for the parental care strategy each individual adopted at its first nest and irrespective of the parental care strategy adopted at subsequent nests (separately for each individual:

**Table 5.3** The reproductive payoff matrices. FC = Female Care, FD = Female Desertion, MC = Male Care, MD = Male Desertion. Capitalized letters above the diagonal indicate the payoffs for the female, lower-case letters below the diagonal line the payoffs for the males. We assume that unilateral desertion yields a higher payoff for both males and females than biparental care. (a) generic version; the arrows correspond to the payoff relationship B > A and b > a. (b) – (e) four specific scenarios for the different relationships between the payoffs for c and D.

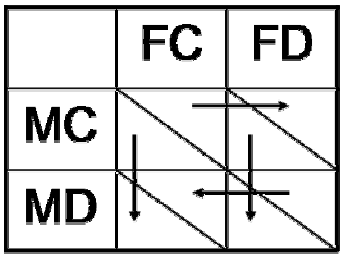
(a)




(b)

c < d and C > D

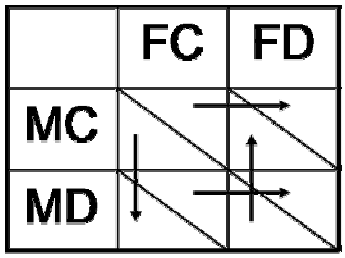
single ESS: female-only care




(c)

c > d and C < D

single ESS: male-only care

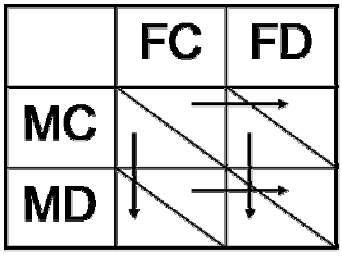



(d)

c < d and C < D

single ESS: biparental desertion

Prisoner's Dilemma

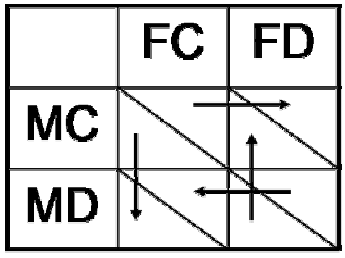



(e)

c > d and C > D

two ESSs: female-only care and male-only care

coordination game (or: Snowdrift game)



$\sum x_1, x_2, \ldots x_i$; x = reproductive success at nest *i*, where the parental care strategy is taken into account for $x_1$ only). If the number of eggs or nestlings was not known for a nest, we used the population mean for a given strategy (i.e. male-only care: 3.67 eggs, 2.22 nestlings; female-only care: 5.80, 3.07; biparental desertion: 2.93, 0.00; $n_{eggs}$ = 371 nests, $n_{nestlings}$ = 194 nests; this study). We used estimated reproductive success for 77 clutches out of a total of 339 clutches (i.e. 22.7% of clutches) and for 33 out of 336 broods (9.8%).

Table 5.2 provides the reproductive output for the different parental care strategies and for males and females separately to illustrate the breeding biology of penduline tits. As we discussed above, the number of eggs and nestlings in male-only cared and biparentally deserted nests is smaller than that in female-only cared nests. Also, once a male cared for the offspring, he does not produce any more offspring in the subsequent nests in the same breeding season, whereas caring females are known to produce up to two clutches in a breeding season.

## GAME-THEORETIC ANALYSIS

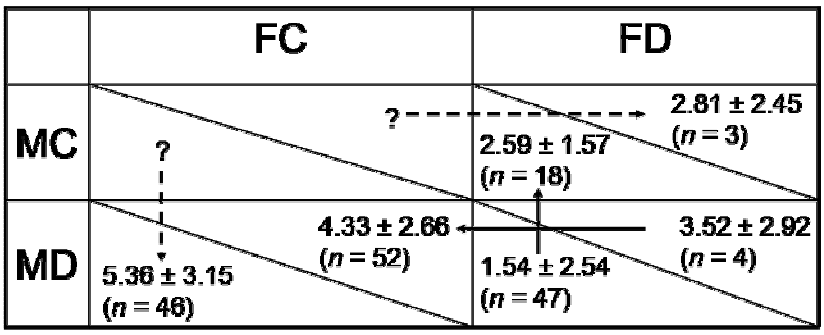
We make the assumption that penduline tits play a single-shot game with only two pure strategies: care or desert. This is a realistic assumption, because pairs rarely produce multiple clutches together and thus reciprocity with tit-for-tat strategies is unlikely (see above; Axelrod & Hamilton 1981; *Chapter IX*). In Table 5.3a we give the possible payoffs for all combinations of strategies played by males and females following the above assumption. Random payoffs would produce mixed strategies, which would include biparental care. In our population, however, we have not observed any case of biparental care (incubation and brood care; *n* = 534 nests; *Chapter VIII*), therefore, the fitness payoff for biparental care (A and a) could not be estimated. Instead, we assume that unilateral desertion of a caring partner always yields a higher fitness payoff than caring (i.e. B > A and b > a). For both sexes, uniparental care yields a higher reproductive payoff than biparental desertion (Table 5.4a), and also the relatively high payoff (5.4 ± 3.2 nestlings) for males in case of unilateral desertion further supports our assumption of b > a.

### *Are Penduline Tits Caught in a Prisoner's Dilemma?*

Cooperative behaviour is often not evolutionarily stable against strategies exploiting the cooperation of others. This may lead to no cooperation at all, a situation exemplified by the Prisoner's Dilemma (PD). In this game, each player may either

**Table 5.4.** The reproductive output of males and females over the breeding season depends on the parental care strategy adopted at their first nest in the breeding season: (a) seasonal mean number of nestlings ± SD. *n* is the number of nests. A male that cared for the offspring at his first nest in the season produced more nestlings than a male whose first nest was biparentally deserted ($U = 257.5$, $P = 0.009$, $n_{mc} = 18$, $n_{bd(biparental\ desertion)} = 47$, Cohen's $d = 0.878$, $1\text{-}\beta = 0.88$). The number of nestlings for a female that cared for the offspring at the first nest in a season, however, was not significantly different from that for females whose first nest was biparentally deserted ($U = 86.5$, $P = 0.591$, $n_{fc} = 52$, $n_{bd} = 4$, $d = 0.528$, $1\text{-}\beta = 0.17$). This matrix is consistent with that of the coordination game (as in (c)). (b) – (e) are the four possible scenarios for the different relationships between the payoffs for c and D; we provide the percentage of cases where the bootstrapping procedure yielded each of the four scenarios.

(a)

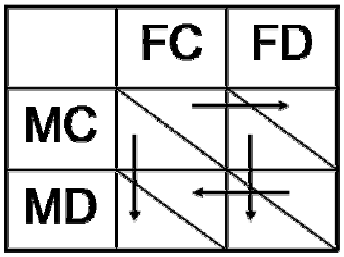



(b)

c < d and C > D

single ESS: female-only care

Probability: 0.230

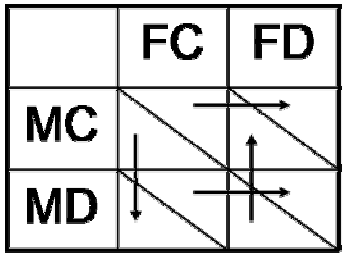



(c)

c > d and C < D

single ESS: male-only care

Probability: 0.277

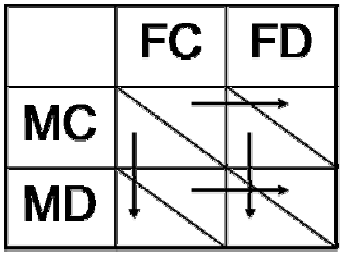



(d)

ESS: biparental desertion

Prisoner's Dilemma

Probability: 0.157

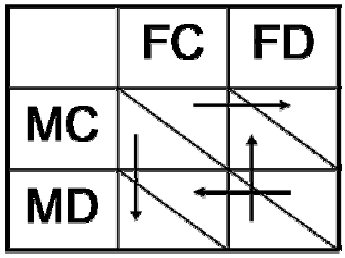



(e)

ESS: female-only care and male-only care

coordination game (or: Snowdrift game)

Probability: 0.336



cooperate, or defect, but the best response is to defect for any action by the opponent (Axelrod & Hamilton 1981; Noë 1990; Nowak & May 1992; McNamara et al. 2004). The payoff matrix of the fitness estimates (Table 5.4a), suggests that desertion is not the dominant strategy and the population as a whole does thus not appear to play the PD. Instead, the snowdrift game provides a better fit. The snowdrift game has been frequently used to study the evolution of cooperation (Doebeli & Hauert 2005; McNamara et al. 2008; Helbing & Yu 2009), although it deals with a symmetric population and has mixed ESSs. Our population, however, consists of two types of players: males and females, i.e. an asymmetric population where not everybody is in the same role. It is a standard result in game theory that in such asymmetric games an ESS can never be a mixed strategy.

The behaviour of penduline tits is most consistent with the coordination game, which has two ESSs instead of mixed strategies. These are the two ways of unilateral cooperation: male-only care and female-only care. Each of these ESSs may be viewed as a convention, where the asymmetry (male versus female) is used to settle the conflict. Although the conflict is settled, once one of these ESSs is reached, the problem remains which of the two ESSs will be reached in the course of evolution. This is where cooperation is coordinated: every player agrees that unilateral cooperation would be best, but each player has a preference for one of the two ESSs. This kind of game has been analyzed in the game theoretical literature under the name 'Battle of the Sexes' (Luce & Raiffa 1957).

*Can Reproductive Payoffs Predict Parental Care?*

Although at a population level penduline tit parents may be playing the coordination game, individual pairs of birds may not always play the same game, but some, for instance, may play the PD. There are four cases to consider for the relationship between c and d and between C and D (Table 5.3). All these will lead to different types of game (Table 5.3b-e) and such pairs may find themselves in

(1) a game where female-only care is the only ESS ($c<d$ and $C>D$);
(2) a game where male-only care is the only ESS ($c>d$ and $C<D$);
(3) a PD game where biparental desertion is the only ESS ($c<d$ and $C<D$); or
(4) a coordination (or snowdrift) game where they are confronted with a coordination problem, since the game has two ESSs ($c>d$ and $C>D$).

To estimate the probability for each scenario, we bootstrapped the individual fitness payoffs (number of nestlings) using field data for each male and female that adopted a given strategy at their first nest (Table 5.4a) 5000 times. We then calculated the percentage of cases where c>d, c<d, C>D, and C<D (Table 5.3) and from that we calculated the probability for each scenario as follows (Table 5.4):

(1) Prob(female-only care) = Prob(c<d) * Prob(C>D) = 0.36 * 0.58
(2) Prob(male-only care) = Prob(c>d) * Prob(C<D) = 0.64 * 0.42
(3) Prob(biparental desertion) = Prob(c<d) * Prob(C<D) = 0.36 * 0.42
(4) Prob(coordination game) = Prob(c>d) * Prob(C>D) = 0.64 * 0.58

When two individuals find themselves in a one-shot coordination game they have to bargain about the outcome. It is conceivable that bargaining does not necessarily result in one of the two ESSs (FC and MC). If the bargaining process fails, biparental desertion might also occur. Although we did not model the bargaining process, we attempted to make predictions on the probabilities of the various outcomes. We applied two solution concepts for coordination games from classical game theory: payoff dominance and risk dominance. A payoff dominant equilibrium will be chosen when the payoff is larger than (or at least equal to) the alternative strategies available to the players. Risk dominant strategies on the other hand are based on the potential losses a player may have to incur when deciding on a strategy. A strategy will thus be risk dominant when the product of the deviation losses for a pair at a given strategy is larger than the alternative (Harsanyi & Selten 1988).

Payoff and risk dominance of the payoffs for the various strategies was determined applying the requirements in Table 5.5. The risk dominance depends, among others, on the payoff values under biparental care. To calculate the latter term, we varied 'a' between $0.2 \leq a \leq 10$ to get $A = B - \mu b + \mu a$, where $\mu = (B-A)/(b-a) = (C-D)/(c-d)$, which follows from equalizing the requirements of risk dominance: $(c-d)*(B-A) = (b-a)*(C-D)$. In these equations b, c, d, B, C, and D are given by the observed payoffs (Table 5.4a). Given our assumption of $b > a$ and $B > A$, to calculate the predicted pattern of care strategies applying risk dominance we set $a = 5.2$ (i.e. $a < b$, where $b = 5.36$) and, accordingly, $A = 2.7$ (i.e. $B > A$, where $B = 2.81$). These approximate the most conservative, but realistic, values under the assumptions $b > a$ and $B > A$.

**Table 5.5** Requirements for payoff dominance and risk dominance of the parental care strategies at the individual level. Payoff dominance is applied to determine the parental care strategy based on the individual payoffs and to resolve the coordination game. If payoff dominance is not decisive in the coordination game, risk dominance may be applied. BD = biparental desertion, FC = female-only care, MC = male-only care

| | payoff dominance of individual payoffs | payoff dominance in coordination game | risk dominance decisive |
|---|---|---|---|
| BD | c < d & C < D | | |
| FC | c < d & C > D | b > c & C > B | (c-d)*(B-A) < (b-a)*(C-D) |
| MC | c > d & C < D | b < c & C < B | (c-d)*(B-A) > (b-a)*(C-D) |

Using the bootstrapped individual payoffs, we found that 66.4% of variation in parental care (male-only care, female-only care or biparental desertion) is directly explained by the reproductive payoffs whereas 33.6% of variation remained unexplained, and these pairs thus ended up in the coordination game (Table 5.4). The latter was then resolved applying payoff and risk dominance (Table 5.5). Payoff dominance led to the following prediction of parental care, which, although significantly different, approached the observed pattern of care in nature (between brackets): 45.4% female-only care (50-70%), 28.0% male-only care (5-20%), and 26.6% biparental desertion (30-40%) (Table 5.1 and 5.4; predicted versus observed pattern of parental care in Hungary: $\chi^2 = 72.21$, $P < 0.001$, $n = 534$ pairs).

Applying risk dominance (with A = 2.7, and a = 5.2) did not improve the fit of the predicted pattern to observed percentages (female-only care: 48.5%, male-only care: 38.5%, and biparental desertion 12.9%), but rather caused a larger deviation from the natural pattern of care (Table 5.1 and 5.4; $\chi^2 = 371.56$, $P < 0.001$, $n = 534$ pairs).

## DISCUSSION

*Are Penduline Tits Caught in a Prisoner's Dilemma?*

To our knowledge, this is the first paper using extensive data collected in the field to explore to what extent the PD may resemble the decision making process over parental care. Despite the immense theoretical interest, there are only few studies, where the PD is supported by field data, in which case some adjustments, such as repeated interactions with tit-for-tat strategies (Iterated PD), to the 'original' single-shot PD, where defection is the only ESS, seem to be essential (Milinski 1987; Legge 1996; Hugie & Lank 1997). The only empirical support for the single-shot PD comes

from the communally breeding Pukeko, *Porphyrio porphyrio*, where communally breeding individuals are at a reproductive disadvantage compared to pairs (Craig 1984). The territorial interactions in that study, however, did not consist of strict two-person games.

Only rarely have such game theoretical models been directly applied to empirical data. Balshine-Earn and Earn (1997) presented a game-theoretic model parameterized with empirical data to investigate the parental care strategies observed in the Galilee St Peter's fish, a cichlid that exhibits a variable breeding system. Here, using a species that exhibits intense sexual conflict over care and, likely as a result of that, an unusually diverse breeding system, we show that Eurasian penduline tit parents appear not to play the single-shot PD game to resolve their bargaining process as to who will care for the offspring. Instead, the fitness payoff matrix of the seasonal reproductive success (number of nestlings) is consistent with that of the coordination game. In the coordination game desertion is still predominant, but it is only an ESS if one of the parents stays behind to care. While in the PD cooperation does not persist, it is maintained at an intermediate level in the coordination game (Doebeli & Hauert 2005). Desertion consequently results in two ESSs, female- and male-only care. We thus found no empirical support for the influential PD, despite the high levels of desertion observed in Eurasian penduline tits.

Penduline tits apparently adopt optimal strategies in terms of reproductive payoffs and are not caught in a situation exemplified by the PD, where the strategies may be evolutionarily stable, but may not necessarily result in the optimal payoffs for a given individual (Dawkins 1980; Doebeli & Hauert 2005; McNamara & Weissing 2009). Some assumptions associated to the PD game, especially those related to the social environment such as reciprocity and no exchange of information during each round, may form major limitations to the applicability of the PD to understand the evolution and existence of cooperation in nature (Noë 1990; Legge 1996). The players in a 'natural game' will, for instance, often closely monitor each other obtaining information about the opponent, and will let the decision on a strategy depend on the opponent's behavior (Noë 1990; Heinsohn & Packer 1995; McNamara et al. 1999; McNamara et al. 2002; Stevens & Hauser 2004; *Chapter III*). We believe it would be useful to find more biological systems to empirically test the applicability of the PD, given its immense theoretical interest and at the same time very scarce empirical support (see: Craig 1984; Milinski 1987; Legge 1996; Hugie & Lank 1997; Stevens & Hauser 2004; Doebeli & Hauert 2005).

*Can Reproductive Payoffs Predict Parental Care?*

Using a novel approach applying payoff dominance within a coordination game structure to resolve the bargaining game Eurasian penduline tit parents may play, we show that their diverse breeding system can be largely explained as reproductive payoff based strategies: the predicted pattern of parental care, based on bootstrapped, observed reproductive payoffs approached the pattern of care observed in nature, including the large proportion of biparentally deserted nests. Additionally, at some two third of nests the variation in parental care is directly explained by payoff dominance. For the remaining one third of nests payoff dominance can resolve the bargaining process as described by a coordination game. Applying risk dominance resulted in a prediction of frequencies of parental care that deviated further from the observed pattern than that predicted by payoff dominance. The theory of risk dominance stems from research in economical sciences (Harsanyi & Selten 1988) and is based on rational decision making. The application of risk dominance in biological sciences needs further exploration, but it may assume a too high level of rationality to be applicable to many biological systems.

The significant deviance from the empirically observed pattern of care can presumably largely be ascribed to individual differences, which may consist of either individual quality or environmental differences. An autocorrelation of individuals playing different strategies may cause a consistent bias in the exact payoffs. Attractive males, for instance, may not only desert when their partner stays behind to care for the offspring, but may also desert when the partner already has deserted. A less attractive male may be better off caring for the offspring in the latter case. Balshine-Earn and Earn (1997) also showed in their model that natural variation between individuals and in the environment could promote the existence of the different frequencies of parental care observed (see also: McNamara et al. 2004). A spatial structure of strategies, such as desert in high quality habitats and care in poor habitats, could lead to spatial autocorrelation, potentially confounding the outcome of our analyses. Although such a spatially diverse environment has been suggested to have the potential of harboring multiple coexisting strategies (Nowak & May 1992), we believe the extent to which this may have influenced our results is likely limited, since in earlier studies we did not find that the parental care strategy was related to habitat characteristics (*Chapter VII & VIII*). However, it remains worthwhile to further investigate the possibility as to how, for instance, mating opportunities may

be spatially structured, potentially resulting in a spatial structure of deserting strategies. Similarly, a social structure may influence to what extent individuals are willing to cooperate and care for the offspring. A social network may have important implications on the decision about parental care (McDonald 2007; Voelkl & Kasper 2009).

Such extrinsic variability in behaviour, the reputation of the players in a game, as well as occasional mistakes made by players, are all ways by which the solution of a game may be influenced in a population and by which multiple strategies may be maintained (Selten 1983; McNamara et al. 2004; Ohtsuki & Iwasa 2007; McNamara et al. 2008; McNamara & Weissing 2009). The ramifications of individual qualities, a spatial or social structure, and environmental effects may be investigated using a state-dependent game theoretic model.

In conclusion, we show that, although individual pairs may play various games, the bargaining game over parental care penduline tit parents may play is most consistent with the coordination game. Payoff dominance of the individual reproductive outputs may resolve this coordination game and explains a large part of the variation of in the pattern of care observed in nature.

**Acknowledgements**

We are grateful to all people who helped with the fieldwork in Hungary. We thank the Kiskunság National Park (23864-2-3/2006, 23864-3-2/2007) and Szegedfish Ltd. for permission to carry out fieldwork. The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002-2006) under contract n. 28696. The work was further supported by a University of Bath studentship to REvD; the Hungarian National Science Foundation OTKA (T043390 to TS); a Royal Society Joint Project grant (15056 to TS); and the Leverhulme Trust (RF/2/RFG/2005/0279 to TS).

# CHAPTER VI

## SEX DIFFERENCES IN CARE PROVISIONING DO NOT EXPLAIN FEMALE-BIASED UNIPARENTAL CARE IN THE PENDULINE TIT *REMIZ PENDULINUS*

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*Manuscript under revision for Animal Behaviour*

*Authors' contributions*

**AP**: data collection, statistical analysis, manuscript preparation

**REvD**: study design, data collection, manuscript improvement

**PH**: data collection

**TS**: study design, manuscript improvement

**ABSTRACT**

Parental care is costly, thus evolutionary theory predicts each parent should shift care provisioning to its mate. We investigated sexual conflict over care in a small passerine bird, the Eurasian penduline tit *Remiz pendulinus.* This species exhibits an unusually complex breeding system: sequential polygamy by both sexes, and uniparental care whereby one parent (either the male or the female) deserts during egg-laying, and its mate incubates the eggs and rears the chicks to independence. In a Hungarian population of Eurasian penduline tits, female-only care of clutch and brood was more common than male-only care (47% *versus* 14% of 534 clutches). Moreover, female-cared clutches were larger than those cared for by males. Here we test the parental quality hypothesis, which states that females provide better care than males, and that this selects for more frequent care by the female. We show that neither incubation behaviour nor brood-feeding rates were different between males and females after controlling for initial clutch size, egg-laying date and ambient weather. Consistent with these results, offspring survival and nestling size did not differ between male-cared and female-cared clutches; our results therefore do not support the parental quality hypothesis. We discuss alternative explanations, such as sex differences in costs and benefits of offspring desertion, which might have led to the observed female-biased parental care in Eurasian penduline tits.

## INTRODUCTION

Sexual conflict theory suggests males and females should adopt strategies that maximize their own reproductive success, regardless of the interest of their mate (Parker 1979; Lessells 1999). Since the interests of males and females over reproduction are often different (for instance, the optimal number of mates is often higher for males than for females), conflict over parental care may emerge such that each parent prefers the other to work harder in provisioning the young (Lessells 1999; Arnqvist & Rowe 2005; Houston et al. 2005). One of the most extreme outcomes of parental conflict (or post-zygotic sexual conflict, Royle et al. 2002) is offspring desertion (Székely et al. 1996). A parent that deserts its offspring offloads the burden of complete parental care to its partner, whereby improving its own survival and future reproductive success (Houston et al. 2005; *Chapter II*).

In animals with uniparental care it is usually the female that cares for the young, although there are abundant exceptions (Clutton-Brock 1991; Székely et al. 1996; Tallamy 2001; Reynolds et al. 2002; Berglund & Rosenqvist 2003). Two fundamental reasons have been suggested to explain why female care is more likely than male care. First, intra- and intersexual selection often results in higher variance in mating success of males than of females. Following on from this, by caring for the young the most successful males in a population would sacrifice higher reproductive success than the most successful females (Queller 1997; Kokko & Jennions 2003). Females, therefore, are expected to enhance the efficiency of their care (parental quality hypothesis; Erckmann 1983; Eckert & Weatherhead 1987), whereas males are expected to evolve to better competitive abilities to access mates. The parental quality hypothesis therefore predicts more frequent and better female care than male care. The second potential reason why female care is more common than male care is that in internally fertilizing organisms such as birds males may be cuckolded. Hence, females more likely rear their own offspring (assuming brood parasitism does not occur), whereas males may not do so. Therefore, the interest of males is often to ensure their mate is fertilized by them (mate guarding), or secure and fertilize many females instead of investing into parental care (Trivers 1972; Queller 1997; Westneat & Stewart 2003; Kokko & Jennions 2008). Consequently, both preceding arguments suggest that female-only care is more likely to evolve than male-only care (McNamara et al. 2002; Kokko & Jennions 2003).

Species with variable breeding systems (sensu Reynolds 1996) offer excellent opportunities to investigate the driving forces behind the evolution of different

patterns of parental care. The Eurasian penduline tit, *Remiz pendulinus*, is a small passerine bird (body mass about 9 g) with a strikingly variable breeding system in which both males and females mate with multiple mates sequentially (up to 7 social mates within a single breeding season). Besides, the clutch and young are cared for by the female (48% - 65%), the male (7% -18 %), or neither parent (28% - 40%, $N$ = 89 - 291 nests in four European populations, (Persson & Öhrström 1989; Franz 1991; Pogány et al. 2008). The high frequency of biparentally deserted clutches, a feature common across all European populations studied to date, indicates intense sexual conflict over parental care (Persson & Öhrström 1989; Valera et al. 1997; Arnqvist & Rowe 2005). The latter conjecture was confirmed by Szentirmai et al. 2007 who quantified the reproductive payoffs of caring and deserting: both male and female penduline tits that deserted their clutch increased their own reproductive success and reduced that of their mate. The penduline tit is the only species as far as we are aware in which the consequences of parental care decisions are mirrored in males and females: what is good for males is bad for females, and vice versa.

Here we test the parental quality hypothesis using a 6-year dataset from a population of Eurasian penduline tits in Hungary. Firstly, we predicted that females should show a greater degree of parental care (incubation and brood provisioning) than males. Secondly, we predicted that nestlings will be larger and chick survival will be higher in nests cared for by females, as a consequence of their greater parental care. Thirdly, we also predicted that males are less able to care for large clutches than females, therefore offspring survival and nestling size should decline more steeply with increasing clutch size in male-cared nests than in female-cared ones.

## METHODS

*Fieldwork*

We studied Eurasian penduline tits at Fehértó, southern Hungary (46º 19’ N, 20º 5’ E) between April and July of 2002 - 2007. Fehértó is an extensive system of 16 fishponds separated by dykes (1321 ha), and penduline tits nest in trees along these dykes. We searched for new nests and checked existing nests approximately every other day throughout the breeding season. Male and female penduline tits were trapped using mist-nets combined with song playback near the nest, or a specially designed nest-trap (Z. Barbácsy, pers. comm.). Birds were ringed with a numbered metal ring from the Hungarian Ornithological Institute, and a unique combination of

three colour rings (A. C. Hughes, Middlesex, UK; for more details on trapping and ringing, see Ethical note, Bleeker et al. 2005; Szentirmai et al. 2007). Penduline tits were sexed according to their sexually dimorphic plumage traits: males have large black masks and are more colourful overall (Kingma et al. 2008).

We recorded incubation and feeding rates at male-cared (MC) and female-cared (FC) clutches between 2005 and 2007 (see Supplementary information). Incubation was recorded at 29 nests (9 MC and 20 FC) during a three hour period (2.83 ± 0.07h, mean ± SE) between the $7^{th}$ and $10^{th}$ day of incubation. If the female had been trapped on the nest during this period, we waited at least one day before recording at that nest (see Ethical note). Sample size for MC is small, because fewer nests are cared for by males than by females (see Results). Recording started at a randomly-selected time between 0700 and 1700 hours. We used a Sony DCR-TR7000 Hi8 and a Panasonic NV-DS28EG mini DV digital recorder mounted on a tripod placed approximately 15m from the nest so that the incubating parents were not disturbed.

We recorded (or directly observed) feeding rates at 30 nests (10 MC and 20 FC) for approximately two and a half hours (2.58 ± 0.11h, mean ± SE) on the $10^{th}$ day after hatching, starting randomly between 0700 and 1700 hours. In 2005 and 2006, the number of feeding visits made at 24 nests (7 MC and 17 FC) was observed from a hide at least 15m from the nest. In 2007, feeding rates were determined at 6 nests (3 MC and 3 FC) using the same video recording system used to monitor incubation. Uniparental care by the male or the female was confirmed at all nests involved in incubation or feeding observations and recordings; only one parent was ever seen to incubate or feed at each nest.

We collected offspring survival data from 150 nests (33 MC and 117 FC) between 2002 and 2007. We estimated clutch size as the number of eggs present on the eighth day of incubation, and brood size as the number of nestlings present 10 days after the first had hatched. We calculated offspring survival at each nest as the proportion of eggs that produced 10-day old nestlings.

To estimate offspring size, we measured body mass and tarsal length of approximately 11-day old nestlings (11.0 ± 0.3 days, mean ± SE) in 90 nests (17 MC and 73 FC) between 2002 and 2004. For these nests we observed clutch size, brood size at hatching and number of chicks 10 days after hatching. The different sample sizes between analyses are due to missing data: for each analysis we included the maximum number of nests to improve statistical power.

*Data processing and statistical analyses*

From the incubation video footage we noted the time when the parent arrived at and left the nest in each incubation bout. We defined arrival and departure as when the parent completely entered the nest and left the nest chamber, respectively, and incubation time was the proportion of time spent inside the nest during the three hour observation period.

We calculated feeding rates at each nest from feeding observations and video recordings. Nest visits without food delivery were rare (no food was delivered in approximately 2% of nest visits at a subset of nests), we therefore used the total number of nest visits as a measure of feeding behaviour. Since brood sizes were different between nests, we calculated feeding frequency per chick per hour.

We controlled for the possible confounding effects of ambient temperature and wind speed on parental behaviour (Conway & Martin 2000) using data from a meteorological station (46º 22' N, 20º 06' E, about 5 km from Fehértó).

We used General Linear Models (GLMs) to examine the independent effects of the sex of the care giving parent, clutch and brood size, ambient temperature and wind speed on incubation rates (arcsine transformed) and feeding rates. In initial models the year of study, time of the day, and first egg dates were also included. The latter was calculated as the number of days from 1 April until the date the first egg of the clutch was laid.

The body mass and tarsus length of 11-day old chicks were averaged for each brood, and we used either mean body mass or mean tarsus length as a dependent variable to investigate the effect of care giving sex on offspring size using GLM. Models of offspring size included the following explanatory variables: care giving sex, exact offspring age (in days), clutch size, number of chicks at hatching and brood size at 10 days after hatching. The effects of caring sex and initial clutch size on offspring survival were investigated using Generalised Linear Models with binomial error distribution.

Assumptions of statistical tests were tested prior to the analyses. Statistical analyses were carried out in R 2.6.1. (R Development Core Team 2005, Vienna, Austria). Non-significant terms were eliminated in a stepwise manner until the final models were reached. We also tested for statistical interactions between care giving sex and the remaining explanatory variables in the models, since these would indicate differential effects of caring sex in regard to specific explanatory variables.

We provide statistics for excluded variables before their exclusion from the model. Mean ± SE and two-tailed probabilities are given, and we rejected the null-hypotheses at $P < 0.05$.

*Ethical note*

We used standard methods as described in our Field Protocol (www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/PT_%20Field%20Guide_1_2.pdf) to search for and check nests, trap and observe penduline tits (Szentirmai et al. 2005a, *Chapter III*). Kiskunság National Park, Hungary, provided the permissions to carry out fieldwork (ref: 577-3/2002; 390-2/2003; 1094/2004; 23864-1-1/2005; 23864-2-3/2006; 23864-3-2/2007). To avoid nest abandonment, we only trapped at nests which were in well-advanced stages (at least in stage C, see Field Protocol). Mated penduline tits were either trapped one day after pairing, or after the eight day of incubation. In both trapping methods (mist-netting or the Barbácsy nest-trap), a trapping trial lasted for less then 30 minutes; if trapping was not successful, we repeated the trial the next day.

Penduline tits are tame and tolerate humans near their nest. Nevertheless, to minimize disturbance, the nests were observed using binoculars from at least 15m every other day to follow breeding status based on behaviour of the parent(s). Clutch size was determined at the eight day of incubation by checking nest content once after the incubating parent left the nest. Based upon these protocols and our extensive fieldwork experience, we believe we recorded the natural behaviour and reproductive success of Eurasian penduline tits.

## RESULTS

*Patterns of care and clutch size*

Out of 534 clutches, the female cared for 253 clutches (47.4%), the male for 72 clutches (13.5%), and 209 clutches (39.1%) were deserted by both parents, whereas biparental care has never been observed. Female-only care was more common than male-only care (binomial test using 325 uniparental clutches and 0.5 expectation, $P < 0.001$).

Females cared for larger clutches (5.8 ± 0.1 eggs, $N = 164$ clutches) than males (3.6 ± 0.2 eggs, $N = 50$ clutches; $t$ test: $t_{212} = 10.235$, $P < 0.001$). Clutch size declined over the breeding season (least squares linear regression: $b = -0.356$, $t = 5.555$, $P < 0.001$). Since earlier studies found seasonal trend in parental care (male-cared clutches are initiated later than female-cared ones; see Persson & Öhrström 1989; Szentirmai et al. 2005a; Pogány et al. 2008), the different dates of nest initiations could be responsible for clutch size differences between the sexes. However, female-cared clutches remained significantly larger when first egg date was included in the GLM (Table 6.1). The non-significant interaction term between care giving sex and first egg date suggests that the seasonal decline in clutch size was

comparable between male-cared and female-cared clutches (Table 6.1), therefore this interaction was not included in the final model.

**Table 6.1** General Linear Model of clutch size (response variable) in Eurasian penduline tits (214 clutches: 164 female-cared and 50 male-cared clutches). All interactions between Care giving sex, Year and First egg date were non-significant, and thus they were excluded from the final model ($R^2$ = 0.457, $F_{7,206}$ = 24.761, $P$ < 0.001).

| | ***F*** | ***DF*** | ***P*** |
|---|---|---|---|
| Explanatory variables in the final model | | | |
| Year | 6.585 | 5 | < 0.001 |
| First egg date | 48.200 | 1 | < 0.001 |
| Care giving sex | 92.200 | 1 | < 0.001 |

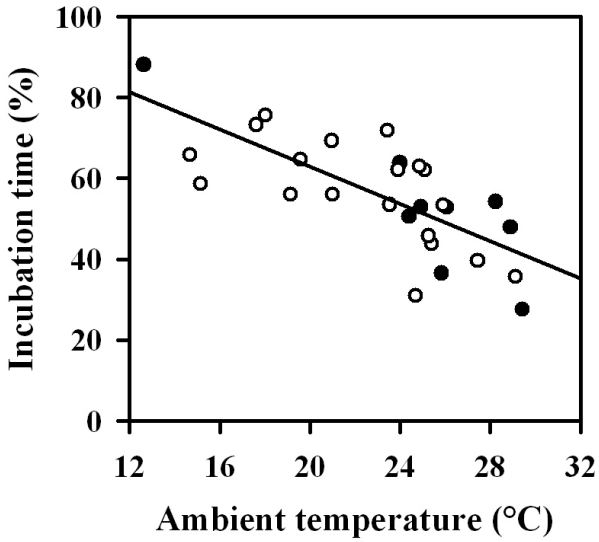


**Figure 6.1** Incubation in Eurasian penduline tits in relation to ambient temperature ($N$ = 28 clutches: 9 male-cared and 19 female-cared nests; least squares linear regressions: $b$ = -0.734, $t$ = 5.512, $P$ < 0.001). Filled symbols: male-only care, open symbols: female-only care.

*Incubation behaviour and chick feeding*

Males and females did not differ in the time they spent on incubation, and we found no difference between the sexes in response to any explanatory variables (Table 6.2). Both males and females increased the proportion of time they spent incubating as ambient temperature decreased (Fig. 6.1, Table 6.2).

Similarly, there was no difference between males and females in brood feeding rates (Table 6.2). Feeding rates per nestling decreased with brood size (Fig. 6.2), and varied between years. No other explanatory variable was significant (Table 6.2).

**Table 6.2** General Linear Model of incubation and feeding behaviour (response variables) in Eurasian penduline tits. Incubation data are from 28 clutches (9 male-cared and 19 female-cared clutches: one female with extreme behaviour was excluded) and feeding data are from 30 clutches (10 male-cared and 20 female-cared clutches). Interactions between Care giving sex and other explanatory variables were non-significant, and thus excluded from the final models (incubation time, $R^2 = 0.548$, $F_{1,26} = 31.471$, $P < 0.001$; feeding rate, $R^2 = 0.460$, $F_{3,26} = 7.394$, $P < 0.001$).

| **Incubation time (proportion of time inside the nest)** | ***F*** | ***DF*** | ***P*** |
|---|---|---|---|
| Explanatory variables in the final model | | | |
| Temperature | 31.471 | 1 | < 0.001 |
| Excluded variables | | | |
| Care giving sex | 0.379 | 1 | 0.544 |
| Wind speed | 0.039 | 1 | 0.846 |
| Clutch size | 0.415 | 1 | 0.526 |
| First egg date | 0.241 | 1 | 0.628 |
| Time of day | 1.968 | 1 | 0.173 |
| Year | 1.003 | 2 | 0.382 |
| **Feeding rate (feeds per chick per hour)** | | | |
| Explanatory variables in the final model | | | |
| Brood size | 6.509 | 1 | 0.017 |
| Year | 7.836 | 2 | 0.002 |
| Excluded variables | | | |
| Care giving sex | 0.084 | 1 | 0.774 |
| Temperature | 0.375 | 1 | 0.546 |
| Wind speed | 0.404 | 1 | 0.531 |
| First egg date | 0.178 | 1 | 0.676 |
| Time of day | 0.330 | 1 | 0.570 |

*Offspring survival and size*

Female-cared clutches, originating from larger initial clutch size (see above), produced more nestlings (3.6 ± 0.1 chicks, $N = 117$ nests) than male-cared ones (2.6 ± 0.2 chicks, $N = 33$ nests; $t$ test: $t_{148} = 3.645$, $P < 0.001$). However, there was no difference in offspring survival between male-cared and female-cared clutches, after controlling for year, initial clutch size and first egg date (Table 6.3). Offspring survival declined more steeply in response to initial clutch size in male-cared clutches than in female-cared ones (as shown by a weakly significant care giving

sex * clutch size interaction when analysing the full dataset; Fig. 6.3, Table 6.3). The latter interaction, however, appears to be driven by an influential data point in MC clutch size of eight eggs, because by restricting the analysis to 1-6 eggs, the interaction is no longer significant (Table 6.3).

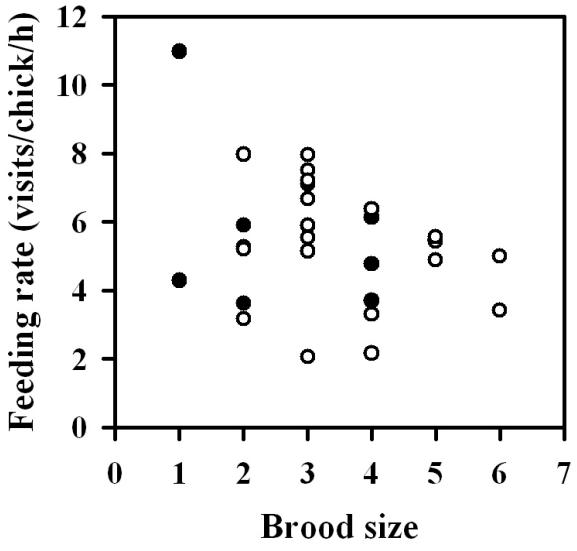


**Figure 6.2** Feeding rates at Eurasian penduline tit nests in relation to brood size (least squares linear regressions: $b$ = -0.303, $t$ = 1.680, $P$ = 0.104). Filled symbols: male-only care, open symbols: female-only care.

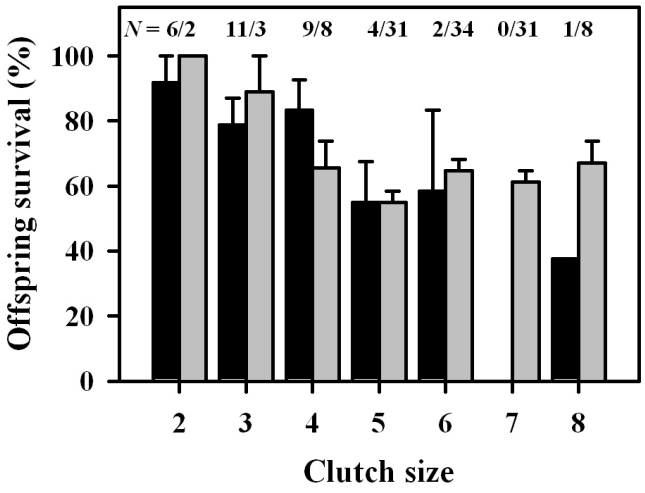


**Figure 6.3.** Offspring survival in Eurasian penduline tits in relation to clutch size (least squares linear regression: $b$ = -0.293, $t$ = 3.725, $P$ < 0.001). Offspring survival is the percentage of eggs that produced 11-day old nestlings, with the number of clutches given above each bar and +1 SEM shown by error bars. Filled bar: male-only care, shaded bar: female-only care.

There was no difference in nestling size between male-cared and female-cared broods (Table 6.4), although chicks' body mass decreased with first egg date and with brood size at hatching (Table 6.4). Both body mass and tarsus length increased with chick age, and were different between years (Table 6.4).

## DISCUSSION

Eurasian penduline tits exhibit one of the most dynamic breeding systems in birds whereby only one sex, usually the female, provides care to the clutch and brood. This may have evolved if females provide better parental care than males. We tested this

hypothesis by comparing patterns and consequences of parental care between male-cared nests and female-cared nests, but contrary to the predictions we found no differences between the sexes in incubation rate, nestling feeding rates, or offspring size and survival.

**Table 6.3** Generalised Linear Model with binomial error of offspring survival in Eurasian penduline tit clutches. Offspring survival was the proportion of eggs that produced 10-day old nestlings. We provide two models: model 1 includes all 150 nests where we collected survival data during our study period (Offspring survival 1: 33 male-cared and 117 female-cared clutches) whereas in model 2 we restricted the dataset to those 110 nests that contained 1-6 eggs (see text; Offspring survival 2: 32 male-cared and 78 female-cared clutches).

| **Offspring survival 1** | $\chi^2$ | ***DF*** | ***P*** |
|---|---|---|---|
| Explanatory variables in the final model | | | |
| Clutch size | 5.295 | 1 | 0.021 |
| First egg date | 9.940 | 1 | 0.002 |
| Year | 11.348 | 5 | 0.045 |
| Care giving sex | 2.178 | 1 | 0.140 |
| Care giving sex * Clutch size | 3.861 | 1 | 0.049 |
| **Offspring survival 2** | $\chi^2$ | ***DF*** | ***P*** |
| Explanatory variables in the final model | | | |
| Clutch size | 9.596 | 1 | 0.002 |
| First egg date | 6.820 | 1 | 0.009 |
| Excluded variables | | | |
| Year | 7.771 | 5 | 0.169 |
| Care giving sex | 0.954 | 1 | 0.329 |
| Care giving sex * Clutch size | 3.023 | 1 | 0.082 |

Why do female penduline tits provide care for more clutches than do males? We suggest three explanations. First, the benefit of desertion may be higher for the male than for the female. Even though in penduline tits either sex may increase its reproductive success by desertion (Szentirmai et al. 2007), the relative costs and benefits of desertion may still be different for males and females, so that the net gain may be higher for the male. To investigate this proposition, we need to compare the Bateman-gradients of the sexes i.e. estimate the rate of reproductive success in relation to the number of mates for males and females, separately (Bateman 1948; Arnold & Duvall 1994; Andersson & Iwasa 1996).

Second, even if the benefits of desertion are similar for males and females, males may be better at manipulating females so that the outcome of sexual conflict is in their favour (Gavrilets et al. 2001). For instance, recent field observations and experiments showed that female penduline tits prefer males with large black facial masks (Pogány & Székely 2007; Kingma et al. 2008), and male penduline tits with a large mask deserted more often than males with a small mask (*Chapter IX*). Therefore, mask size may act as a manipulative trait by which males with large mask entice their partner to care – with an outcome similar to differential allocation in which females are willing to care more for their young if an attractive (or high quality) male sired them (Burley 1988; Sheldon 2000). Males may also detect egg-laying of their mates, and this gives them the upper hand in deciding first whether care for the clutch or desert (Valera et al. 1997).

Third, males may provide less care because they are unsure about paternity (Queller 1997). Using molecular markers, we are currently investigating the rate of extra-pair paternity in penduline tits; Schleicher et al. (1997) found 6.9% of extra-pair paternity (EPP) whereas our single-locus fingerprinting suggests 16% EPP, and no difference between male-only and female-only clutches (Mészáros et al. in prep).

Male and female penduline tits spent comparable time on incubation, and they fed their young at similar frequencies. Incubation time did not differ although males incubated on average 40 % smaller clutches. Besides, and in contrast with other studies, the number of eggs did not influence incubation effort within either sex, suggesting that incubation time in penduline tits is not adjusted to clutch size (Haftorn & Reinertsen 1985; Dobbs et al. 2006). A possible explanation for this discrepancy is that penduline tits have well-insulated nests (Szentirmai et al. 2005b) compared with many tree-hole (or nest-box) nesting passerines investigated by the aforementioned researchers. Moreover, penduline tits gain body mass during incubation, suggesting that incubation does not have an excessive energetic cost (Bleeker et al. 2005). Similar to females, male penduline tits also develop a brood patch during incubation (Cramp et al. 1993), and this further decreases the opportunity for parental quality differences during incubation. Brood size, in contrast with incubation time, constrained per chick feeding frequency, however the decrease was comparable between male-cared and female-cared broods.

**Table 6.4.** General Linear Model of body mass and tarsus length of 11-day old nestlings. Data was collected in 90 clutches (17 male cared clutches/broods and 73 female-cared). All Care giving sex * Explanatory variable interactions were non-significant and excluded (final models: body mass, $R^2$ = 0.392, $F_{5,84}$ = 10.831, $P$ < 0.001; tarsus length, $R^2$ = 0.469, $F_{3,86}$ = 25.273, $P$ < 0.001).

| **Offspring body mass** | ***F*** | ***DF*** | ***P*** |
|---|---|---|---|
| Explanatory variables in the final model | | | |
| Year | 8.600 | 2 | < 0.001 |
| First egg date | 5.121 | 1 | 0.026 |
| Offspring age | 19.464 | 1 | < 0.001 |
| Brood size at hatching | 12.368 | 1 | < 0.001 |
| Excluded variables | | | |
| Care giving sex | 0.459 | 1 | 0.500 |
| Initial clutch size | < 0.001 | 1 | 0.987 |
| Current brood size | 1.192 | 1 | 0.278 |
| **Offspring tarsus length** | | | |
| Explanatory variables in the final model | | | |
| Year | 29.069 | 2 | < 0.001 |
| Offspring age | 17.683 | 1 | < 0.001 |
| Excluded variables | | | |
| Care giving sex | 0.014 | 1 | 0.906 |
| First egg date | 0.068 | 1 | 0.794 |
| Initial clutch size | 0.018 | 1 | 0.893 |
| Brood size at hatching | 0.025 | 1 | 0.876 |
| Current brood size | 0.042 | 1 | 0.838 |

We found no differences in offspring survival (nor in average chick size and body mass) between male-cared and female-cared clutches, although the latter still produced more offspring as a result of sex differences in initial clutch size. Offspring survival decreased with advance of the breeding season, which may reflect seasonal changes in food availability. An alternative explanation for the seasonal variation is provided by the optimal annual routine hypothesis (McNamara & Houston 2008), as parents may trade-off parental efforts in favour of their own reserves before the autumn migration starts. In line with the annual routine hypothesis, clutch size also decreased with advance of the breeding season, as in many other bird species (Rowe et al. 1994). Sexes differed in offspring survival in response to initial clutch size

when analysing our full dataset, however, this sex difference appeared to be driven by an influential data point in male-only cared clutch size of eight.

Although male and female parental care was not different for both indirect and direct measures of parental quality, it is possible that some consequences of the caring sex might have remained hidden. For instance, quality of food (size or type of prey) given to offspring might have been different between male-only and female-only nests. As a consequence, recruitment (or lifetime reproductive success) of the offspring could be different between male-cared and female-cared broods.

The small clutch sizes of male-only cared nests are probably due to female desertion and thus 'incomplete' clutches (Persson & Öhrström 1989). The deserted male then has two options: desert as response, or care for the clutch. The males we investigated in this study took the second option. It would be very interesting to manipulate desertion behaviour experimentally by removing one parent randomly during egg-laying, and also to manipulate clutch-size. These experiments may clarify whether males would be able to provide care for larger clutches comparable to females as suggested by our results. Furthermore, it remains to be explored how the long-term costs of care may vary between males and females; if care reduces male survival more than that of females, one would expect to see female-biased parental care in penduline tits.

In conclusion, our results do not support the argument that female-biased uniparental care in Eurasian penduline tits is due to females providing better care than males. Further studies may reveal whether the observed female-biased care is driven by sex difference in the benefits (or costs) of offspring desertion. We conjecture that in Eurasian penduline tits either the males have higher mating opportunity (and thus their reproductive output increases more steeply with desertion) than that of the females, or by providing care the males pay higher mortality cost in future years than do females.

**ACKNOWLEDGEMENTS**

The research leading to these results has received funding from the European Community's Sixth Framework Programme (GEBACO, FP6/2002-2006) under contract no. 28696. This research was also supported by the Hungarian Scientific Research Foundation (OTKA T031706 and T043390). Permission to work at Fehértó fishpond was provided by Kiskunság National Park (Hungary) and Szegedfish Kft. (Hungary). We are grateful to István Szentirmai, Gábor Bakacsi, Béla Tokody, Lídia Mészáros and all the field-workers for their assistance. We thank András Kosztolányi, Zoltán Barta and Freya Harrison for their comments which improved the manuscript. Weather data was provided

by the Hungarian Meteorological Service. We also acknowledge the insightful comments of Ian Stewart, an anonymous Reviewer and the Editor.

# CHAPTER VII

# THE INFLUENCE OF HABITAT STRUCTURE ON SEXUAL CONFLICT OVER CARE IN PENDULINE TITS

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*Ardea 96: 3-11*

*Authors' contributions*

**REvD**: study design, data collection, statistical analysis, manuscript preparation

**AEP**: data collection

**IS**: study design, data collection, manuscript improvement

**TS**: study design, manuscript improvement

**JK**: manuscript improvement

**ABSTRACT**

Spatial and temporal distributions of resources and habitats often influence breeding systems. These influences are particularly relevant in those species which exhibit variable breeding systems. We studied such a species, the Penduline Tit *Remiz pendulinus*. This small passerine bird has sequential polygamy by both sexes, and evidence suggests intense sexual conflict between males and females over care. We estimated habitat structure by scoring the vegetation important for nest building and foraging in the immediate surroundings of the nest. Using four principal components we show that sites with more abundant vegetation are occupied earlier than sites with sparse vegetation. However, habitat structure does not predict mating success or reproductive success, and it neither predicts which parent (the male, the female or both) deserts the clutch. We therefore suggest that habitat structure does not have a direct effect on reproductive success or on the resolution of sexual conflict in Penduline Tits. Specific aspects of habitats, such as food and nest material availability, remain to be tested.

## INTRODUCTION

The distribution and availability of resources often influence breeding systems in birds. The degree of spatial homogeneity of distribution of resources, for instance, influences the way males establish territories and subsequently the number of females they may obtain (Verner 1964; Verner & Wilson 1966; Orians 1969; Bennett & Owens 2002). Abundant food often promotes polygamy (Davies 1991; Andersson 2005). However, the link between food availability and breeding system is likely to be more complicated than often anticipated (Kosztolányi et al. 2006). For instance, interference competition, the competitive interaction between individuals over resources, appears to play an important role in the distribution of individuals (Kosztolányi et al. 2006; Vahl 2006). These distributions in turn, may affect mate availability and thus the costs and benefits of desertion (Emlen & Oring 1977; Alatalo et al. 1981; Davies 1989; Székely et al. 1999).

One of the drivers of breeding system evolution is sexual conflict; this emerges if the evolutionary interests of males and females over reproduction are different (Davies 1992; Birkhead & Parker 1997; Houston et al. 2005; Thomas et al. 2007). In species where there is sexual conflict over care provisioning, such as the Dunnock *Prunella modularis* (Davies 1992, Kentish Plover *Charadrius alexandrinus* Székely et al. 1999, or the Snail Kite *Rostrhamus sociabilis* Beissinger 1987), food abundance may play an important role in resolving this conflict. In polygamous species, for instance, high food availability may allow females to lay subsequent clutches, with males having to care for the young (Andersson 2005). Furthermore, high food availability may promote the existence of sexual conflict as it promotes the sufficiency of uniparental care for the survival of offspring, opening up the possibility for one parent, either male or female, to desert. In the Snail Kite, for instance, mate desertion occurs more frequently when food is abundant (Beissinger 1987). Which parent deserts may depend on ecological factors in species with plastic expression of alternative breeding strategies (Emlen & Oring 1977; Davies et al. 1995; Székely et al. 1999; Wysocki 2004; Magellan & Magurran 2006). For instance, if there are many unpaired males available in a population, the female may more likely desert than the male, if either parent can care for the offspring on its own. Similarly, if the remating opportunities are better for males, males are more likely to desert.

Here we investigate whether habitat structure, as a proxy measure for food and nest material availability, influences the outcome of sexual conflict over parental

care in Penduline Tits *Remiz pendulinus*. Sexual conflict in this small passerine is exhibited by parental care always being carried out by one parent, either by the male (5-20%), or by the female (50-70%). In addition, some 30-40% of clutches is deserted by both parents during egg-laying (Persson & Öhrström 1989). This pattern of parental care suggests intense sexual conflict over care. Szentirmai et al. (2007) recently showed that both males and females gained by deserting, but both pay a cost if their mate does so. Furthermore, the process of desertion is rapid: biparental desertion takes place within one or at most within two days, suggesting an arms race for deserting first (*Chapter III*).

Parental care is costly in terms of survival, energy and remating opportunities (Dijkstra et al. 1990; Webster 1991; Székely et al. 1996; Magrath & Komdeur 2003). Therefore each parent is expected to shift care to its mate (Houston et al. 2005). One may expect that habitat structure influences the observed pattern of care. Given that clutches cared for by females are significantly larger (5.8 ± 1.3 eggs) and are thus likely to have a higher energy demand than clutches cared for by males (3.5 ± 1.4 eggs, *Chapter III*), female-only care is expected to be more prevalent in food rich habitats, whereas male-only care may be more frequent in habitats with low food availability. Food rich habitats will be beneficial for both male and female: the female is more likely to successfully raise the young. This would offer the male the opportunity to desert. However, if there is little food available, the female might try to pre-empt desertion by the male, attempting to force him to care (Lazarus 1990; Barta et al. 2002). We would therefore predict that males would occupy food-rich habitats first after returning from their wintering grounds. A similar argument can be made in regards to nest material availability: female-only care is expected in habitats offering more nest material. Females are likely to care for males that spend more time on nest building and also for males that build large nests (Szentirmai et al. 2005a). This offers the males the opportunity to desert and avoid the costs of parental care. In sum, males are expected to occupy habitats that have dense vegetation and abundant nest material first.

## METHODS

*Study species*

The Penduline Tit is a small passerine (body mass about 9-10g), and has a widespread distribution across Europe and Asia. It exhibits sequential polygamy by both sexes, and both male and female may have up to six partners in a single

breeding season (Persson & Öhrström 1989; Houston et al. 2005; Szentirmai et al. 2007).

We studied Penduline Tits in Hungary (see below). Penduline Tits were ringed with a unique combination of three colour-rings and one numbered metal ring (Bleeker et al. 2005). Standard morphometric measurements were taken. The size of the eye-stripe (the 'mask') of the birds, signalling attractiveness, was measured using digital photographs and analysed using Adobe Photoshop 7.0 (Kingma et al. 2008). In total there were 177 and 158 nests in 2004 and 2005, respectively. Of the 62 males and 44 females we colour-ringed in 2004, only three males and one female were re-sighted in 2005. We searched the complete study area for new, unpaired, nest-building males, and visited each active nest (i.e. in nest-building, egg-laying, incubating, or feeding phase) nearly every other day (*Chapter III*). At each nest the following dates were recorded: (i) Nest initiation date. The exact date could be determined for nests found at the date of initiation of nest-building, when only a small amount of material is woven around a twig (Cramp et al. 1993). For nests in later stages of development the nest initiation date could be estimated using nests found on the day of nest-building initiation as a reference (Szentirmai et al. 2005a). (ii) Date of pair formation. A male was considered to be mated as soon as the pair was seen copulating near the nest, or building the nest together. (iii) Date of desertion. A nest was considered to be deserted by one or both of the parents if a male or female (or both) was not seen at the nest for at least 15 minutes for at least two consecutive nest checks. A 15 minute period appears to be enough to establish to presence of a bird at its nest (*Chapter III*). Mating time was defined as the number of days it took a male to attract a mate from the date it started building its nest. Mating success was defined as whether pair formation took place or not. Dates are given as number of days since 1 March in each year.

*Habitat structure*

Data were collected in 2004 and 2005 in Fehértó (1321 ha) in southern Hungary ($46^0$ 19' N, $20^0$ 5' E). From 7 May to 15 June in 2004, and from 18 May to 10 July in 2005 habitat structure was investigated for 48 and 139 nests, respectively. Fehértó is an extensively used fish-farm consisting of large fishponds separated by dikes. The reed beds *Phragmites australis* were along the dikes, and Penduline Tits built their nests in Willows *Salix spp*., Poplars *Populus spp*. and Russian Olives *Eleagnus angustifolius* – the reeds and these trees were used both for foraging and searching

for nest material (Cramp et al. 1993; Darolová & Krištofík 1993; Glutz von Blotzheim 1993; Krištin 1995; Grubbauer & Hoi 1996; Szentirmai 2005). Penduline Tits in our study area mainly foraged on Poplars, Willows and Reed, as also reported by Krištin (1995) and Cramp et al. (1993), but rarely on Russian Olives (A. wa Kang'eri & D.M. Brinkhuizen, unpubl. data). In addition, nestling feeding rates seemed to increase with habitat structure, estimated as the number of trees and coverage of reed within 50m surrounding the nest (see below; A. wa Kang'eri & D.M. Brinkhuizen, unpubl. data). The above trees and reed made up the vast majority of vegetation in our study area. Elder *Sambucus nigra* and Black Locust *Robinia pseudoacacia* also occurred, but they were rarely used for nest building (R.E. van Dijk unpubl. data, Darolová & Krištofik 1993). The remaining vegetation in the area may consist of grass or agricultural lands; both are unsuitable for foraging or collection of nest material by Penduline Tits. There was, however, variation in the constitution of the above vegetation surrounding the different nests in our study site, varying from only a few trees with no more than five percent reed cover to small patches of wood surrounded by wide reed beds.

Habitat structure was scored within a radius of 50m around each nest. The minimum distance between simultaneously active nests is approximately 50m (Glutz von Blotzheim 1993). Within this radius we counted the number of live Poplars, Willows and Russian Olives. A distinction between large and small trees was made as follows: for Poplars, 'small Poplar': trunk circumference at 1.5m height < 60cm; 'large Poplar' trunk circumference at 1.5m ≥ 60cm; Poplars shorter than 1.5m were not counted. For Willows we estimated the percentage of daylight covered by leaves, i.e. canopy density, in combination with the height of the tree was used as follows: 'small Willow': canopy density < 50%, irrespective of tree height, or canopy density ≥ 50% and tree height ≤ 6m; 'large Willow' tree height > 6m. Two observers carried out tree counts in 2004. In 2005 trees were counted by a third observer. Reed coverage in the above area was estimated by drawing a sketch of the vegetation from which coverage was estimated visually to 5% accuracy. All reed estimates were carried out by one observer in both years.

*Data analyses*

Variables describing the habitat structure were multi-collinear, we therefore carried out a principal component analysis (PCA) to extract four principal components (PCs) using varimax rotation with Kaiser normalisation. These four components together

explain 81.2% of the variance in habitat structure (Table 7.1), and they did not differ between the two years of study (Mann-Whitney U; $P > 0.104$, $n = 187$). PC1 mainly accounts for the number of Poplars, PC2 for the number of Russian Olives, PC3 for the number of Willows, and PC4 mainly accounts for reed coverage (Table 7.1).

**Table 7.1.** Factor loadings of each variable, eigenvalue per component and percentage of variance explained by each component of the principal component analysis[a, b].

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Reed coverage | 0.085 | -0.002 | -0.091 | **0.977**** |
| Large Willows | 0.099 | -0.148* | **0.781**** | -0.125 |
| Small Willows | 0.002 | 0.045 | **0.863**** | 0.014 |
| Large Poplars | **0.916**** | -0.010 | -0.032 | 0.010 |
| Smale Poplars | **0.882**** | 0.059 | 0.142 | 0.097 |
| Large Russian Olives | 0.083 | **0.875**** | -0.081 | 0.192* |
| Small Russian Olives | -0.032 | **0.897**** | -0.023 | -0.186* |
| Eigenvalue | 1.643 | 1.597 | 1.392 | 1.052 |
| % variance accounted for | 23.5 | 22.8 | 19.9 | 15.0 |

[a] * Correlation is significant at $\alpha = 0.05$

** Correlation is significant at $\alpha = 0.001$

[b] Absolute values of factor loadings > 0.7 are in bold.

We used these PCs as independent variables, and male behaviour, e.g. date when a male started building its nest, and reproductive success as response variables (Table 7.2). If we assume that the first returning Penduline Tit occupies the higher quality territories first, then one expects a negative association between the PCs and date of nest-building (Aebischer et al. 1996; Currie et al. 2000; Eckerle & Thompson 2006).

To avoid pseudoreplication, only one randomly selected nest of colour-ringed males was included in the dataset – except in the analyses of nest building when the first nest of each individual male was included – and the composition of pairs was always different, unless otherwise stated. Unringed males were excluded from the analyses.

A potential effect of pseudoreplication of nest-sites cannot be excluded. However, given the sample sizes for each separate test and the fact that the data originate from two years, this effect is likely to be small. Moreover, several nests are rarely built in exactly the same nest-site, i.e. the same tree, within a year (R.E. van Dijk & I. Szentirmai, pers. obs.). Between years this is possible, but habitat structure

is likely to change for a given nest-site at a given time in the season between years. Data on mating time were normalised using a log(x+1) transformation. Analyses were performed using SPSS 14.0.0 for Windows. We provide mean ± SD.

**Table 7.2.** Descriptive statistics of response variables.

| | mean ± SD | range | *n* |
|---|---|---|---|
| Date of nest-building initiation | 92.7 ± 21.3 | 34 – 132 | 56 |
| Male mask size ($cm^2$) | 1.36 ± 0.25 | 0.80 – 1.79 | 41 |
| Mating time (days) | 8.3 ± 7.2 | 0 – 35 | 46 |
| Number of eggs | 4.2 ± 1.9 | 1 – 8 | 46 |
| Number of chicks | 3.7 ± 1.6 | 1 – 7 | 29 |
| Hatching success (%) | 68.9 ± 21.2 | 33.3 – 100.0 | 33 |

## RESULTS

Habitat structure influenced the date males started nest-building at a given nest-site (Table 7.3). This was mainly accounted for by PC1 (Fig. 7.1), suggesting that habitats containing a larger number of poplars are selected first by males. The slopes for PC1, PC3 and PC4 were negative (unstandardised regression coefficients $\beta$ = -12.398, -2.622, and -0.446, respectively). The slope for PC2 was positive ($\beta$ = 0.139). Although the individual effects of PC2, PC3 and PC4, were not significant ($P > 0.232$), overall, nest-sites were occupied earlier with increasing values for habitat structure.

**Table 7.3.** Regression ANOVAs investigating the effect of habitat structure (expressed as four PCs; see Table 7.1) on date of nest-building initiation, male mask size, mating time and reproductive success. Separate models were created for each response variable to maximise sample sizes (see Table 7.2).

| | *F* | *P* |
|---|---|---|
| Date of nest initiation | 4.858 | 0.002 |
| Male mask size | 0.391 | 0.814 |
| Mating time | 1.080 | 0.379 |
| Number of eggs | 0.463 | 0.762 |
| Number of chicks | 1.077 | 0.390 |
| Hatching success | 1.096 | 0.378 |

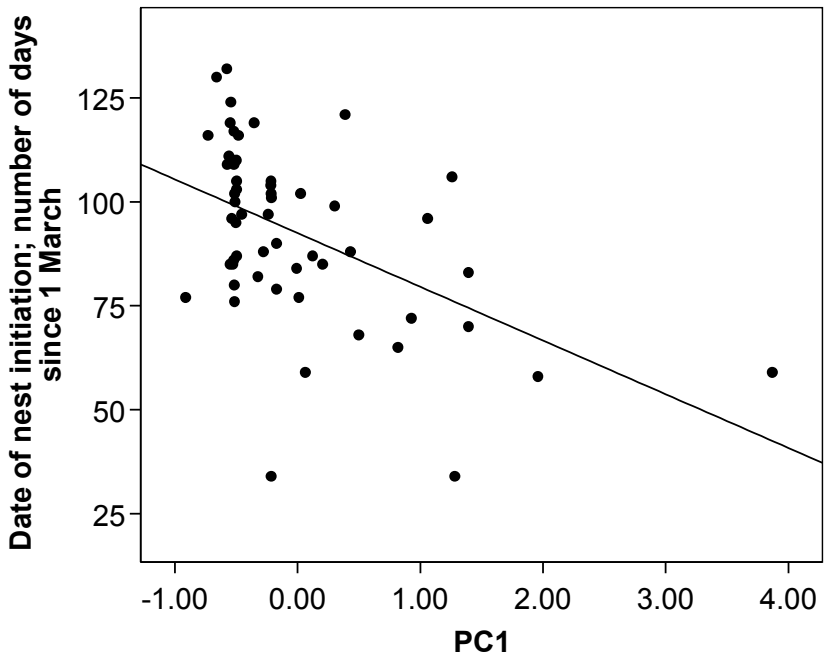


**Figure 7.1.** With an increasing PC1, mainly accounted for by the number of Poplars, nest-sites were occupied earlier in the season (Least Squares Regression; unstandardised regression coefficient $\beta$ = -12.921, $F$ = 18.483, $r^2$ = 0.255, $n$ = 56 nests, $df$ = 1, $P$ < 0.001).

Habitats with a higher vegetation density were not occupied by more attractive males (Table 7.3), which have larger mask sizes (Kingma et al. 2008). Also, habitat structure did not predict reproductive success, as measured by the number of eggs at the eighth day after start of incubation, the number of nestlings at the tenth day after hatching, and hatching success calculated as the percentage of offspring that survived from egg to ten days old nestling (Table 7.3).

At 114 out of 187 nests included in this study across two years the male attracted a female to its nest and egg-laying was initiated (2004: 36 out of 48 nests, 2005: 78 out of 139 nests). We did not find any effect of habitat structure on mating time (Table 7.3), nor on mating success (Table 7.4a).

None of the habitat structure variables predicted whether (a) the female or (b) the male was more likely to care or desert (Table 7.4b and 7.4c).

## DISCUSSION

Consistent with our predictions, we show that habitats consisting of a denser vegetation structure in the immediate surroundings of the nest were occupied earlier in the season. Similar results have been reported for other bird species (Aebischer et al. 1996; Currie et al. 2000; Eckerle & Thompson 2006). However, habitat structure did not affect reproductive success of Penduline Tits (see also

**Table 7.4.** Binary logistic regression models of (a) mating success ($n$ = 70 nests), (b) female care strategy ($n$ = 41 nests), and (c) male care strategy ($n$ = 41 nests) in response to habitat structure. Desertion by the female is defined as female-only desertion and biparental desertion; similar for desertion by male. In both models care was labelled '0' and desertion '1'. All models adequately fit the data (Hosmer-Lemeshow goodness-of-fit; (a) $\chi^2 = 6.217$, $df = 8$, $P = 0.623$; (b) $\chi^2 = 2.615$, $df = 8$, $P = 0.956$; (c) $\chi^2 = 14.979$, $df = 8$, $P = 0.060$). Predicted effect sizes and standard errors are given.

| | Model effect estimate (± SE) | Wald | *P* |
|---|---|---|---|
| (a) | | | |
| PC1 | -0.243 ± 0.236 | 1.062 | 0.303 |
| PC2 | 0.405 ± 0.366 | 1.227 | 0.268 |
| PC3 | -0.029 ± -0.283 | 0.010 | 0.919 |
| PC4 | 0.108 ± 0.300 | 0.129 | 0.719 |
| | | | |
| (b) | | | |
| PC1 | -0.032 ± 0.478 | 0.004 | 0.947 |
| PC2 | 0.100 ± 0.328 | 0.093 | 0.761 |
| PC3 | 1.476 ± 2.709 | 0.297 | 0.586 |
| PC4 | -0.555 ± 0.386 | 2.073 | 0.150 |
| | | | |
| (c) | | | |
| PC1 | -0.537 ± 0.525 | 1.048 | 0.306 |
| PC2 | -0.231 ± 0.345 | 0.449 | 0.503 |
| PC3 | -0.404 ± 0.424 | 0.909 | 0.340 |
| PC4 | 0.435 ± 0.502 | 0.752 | 0.386 |

Darolová & Hoi 1996), nor did it influence the outcome of sexual conflict over nestling provisioning in our study population. The latter result contradicted our expectations: we would expect that the relatively small male-only cared clutches require less food than the larger female-only cared clutches. We would therefore expect more male care in habitats with less suitable vegetation (Krištin 1995). However, the difference in number of nestlings between male- and female-only cared clutches was not significant (2.7 ± 1.1 nestlings for male-only ($n$ = 7 nests) versus 3.8 ± 1.5 nestlings in female-only ($n$ = 7, 39 nests, respectively); Mann-Whitney U = 79.500, $P$ = 0.074). This non-significant difference in number of nestlings may partly explain why Penduline Tits did not adjust clutch size and type of parental care (male-care, female-care or biparental desertion) to habitat structure, given that the nestling-

phase is when food availability is likely to be most crucial in Penduline Tits (Bleeker et al. 2005). However, it should be noted that the sample size for male-only cared nests was small and that the difference in number of nestlings was close to significance. Also, although there was some variation in hatching success (Table 7.3), there was no effect of habitat structure on hatching success. This suggests that food was sufficiently available for larger broods being raised by one parent only. The variation in hatching success may have been due to other factors than habitat structure, such as parental qualities. Nestling survival, calculated as percentage of nestlings that survived from day of hatching till ten days after hatching, is high in our population (78.7% ± 24.3%, R.E. van Dijk, I. Szentirmai, T. Székely, unpubl. data). This again suggests that food is generally sufficiently available. These results on reproductive success versus habitat structure are consistent with the suggestion for Blue Tits *Cyanistes caeruleus* that food limitation during the nestling period might not be the primary force shaping the reproductive output, and that in food rich habitats potential effects on reproductive success are much less pronounced (Tremblay et al. 2003).

We did not find an effect of habitat structure on either mating success or mating time. A line of arguments similar to that described above for reproductive success and provisioning may be followed here. If food and nest material is *generally* very abundant, then a male in a vegetation rich habitat will not have a pronounced mating benefit over a male in a habitat with slightly less vegetation. The idea of generally abundant food and nest material resources fading out a potential effect on mating success and breeding biology is supported by the fact that uniparental care appears to be sufficient for the survival of the offspring and by the low territoriality of the species. However, other possible explanations for our failure to find an effect of habitat structure on the Penduline Tit's breeding biology should not be excluded. For instance, (i) food and nest material availability may vary among habitats with similar vegetation structure. A more direct quantification of food and/or nest material availability may find support for our hypotheses. (ii) We did not find an effect of habitat structure on the breeding biology of Penduline Tits within one population. It may be interesting to compare populations breeding at different sites, with a potential for larger variation in habitat structure.

Although we did not find any influence of habitat structure on the breeding system of Penduline Tits within the two years of study, on an evolutionary timescale the impact may have been prominent (Davies et al. 1995). Although we do not have

precise measurements on food availability, food in our study area seems to be generally abundant (R.E. van Dijk, I. Szentirmai, and T. Székely pers. obs.). Such a high food availability may have promoted the evolution of sexual conflict over care provisioning and subsequent polygamy in this species (Davies 1991; Andersson 2005), since it will facilitate the survival of offspring with uniparental care only. Incidentally, a closely related species of the Eurasian Penduline Tit, the Cape Penduline Tit *Anthoscopus minutus* (Sibley & Ahlquist 1995), lives in a poorer habitat, the South African 'fynbos', and it exhibits facultative cooperative breeding, radically different from the Eurasian Penduline Tits (R.E. van Dijk and T. Székely pers. obs.). In the future it will be interesting to carry out phylogenetic comparative analyses among closely related species, e.g. including Remizidae and Paridae, to investigate the influence of habitat structure on breeding system evolution. Using a detailed phylogeny one may be able to distinguish between two evolutionary trajectories: (i) sexual conflict evolved in food rich habitats, or (ii) species exhibiting sexual conflict spread into food rich habitats. Together with case studies such as the one we present here, this may help to unravel the causes behind variable breeding systems at different taxonomic levels (Davies et al. 1995). Furthermore, experimental manipulation of food and/or nest material availability will be a more direct approach to test its importance in resolving sexual conflict over care.

**ACKNOWLEDGEMENTS**

We thank Dušan Brinkhuizen and Arno wa Kang'eri for collecting data on habitat structure in 2004. Kiskunság National Park and Szegedfish Ltd. kindly permitted us to carry out fieldwork in Fehértó, Hungary. We are grateful to Ken Kraaijeveld and one anonymous reviewer for their constructive comments on our manuscript. The study was financially supported by a Studentship of the University of Bath, the Marco Polo Fonds and the Groninger Universiteitsfonds to REvD, and grants to TS (Hungarian Scientific Foundation OTKA T031706, T043390, The Royal Society 15056 and the BBSRC BBS/B/05788).

# CHAPTER VIII

## PARENTAL CARE STRATEGIES IN EURASIAN PENDULINE TIT ARE NOT RELATED TO BREEDING DENSITIES AND MATING OPPORTUNITIES

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*submitted manuscript*

*Authors' contributions*

**REvD**: study design, data collection, statistical analyses, manuscript preparation

**DMB**: data collection, statistical analyses, manuscript improvement

**TS**: manuscript improvement

**JK**: study design, manuscript improvement

**ABSTRACT**

Breeding density may influence breeding systems in various manners. For instance, the number of potential mates may influence the mating system and parental care strategies. The breeding system of Eurasian penduline tits, *Remiz pendulinus,* involves sequential polygamy by both sexes and uniparental care by either male or female. Additionally, about one third of clutches is deserted by both parents. Mating opportunities and breeding behaviour were studied at two populations: a low-density population (The Netherlands) and a high-density population (Hungary). We expected that higher breeding density is associated with higher incidence of polygamy. However, despite the substantial differences in breeding density and mating opportunities between Hungary and The Netherlands, there was no difference in the frequency of parental care types. We further investigated the plasticity of penduline tit's breeding system using data from five breeding populations in Europe, and found that care patterns were not different between these populations. We conclude that the type of parental care is not related to local breeding density. Long-distance dispersal of penduline tits may mix the gene pool, so that local adaptation cannot possibly occur. Alternatively, proximate mechanisms of breeding system variation (e.g. gene expression) may be non-flexible and thus not adjusted to local conditions.

## INTRODUCTION

Population size and density may influence various aspects of breeding systems. Population densities may be associated with a range of ecological and life-history variables, including competition for nest sites and nest material (e.g. Kokko et al. 2004; Alonzo & Sheldon 2009), and a potential for extra-pair copulations (Widen & Richardson 2000; Komdeur 2001; Mougeot 2004). These may all affect the process of decision making for males and females as to how much to invest into rearing a brood. An increased rate of extra-pair copulations may make males more prone to desert the offspring, as the genetic share they hold in the offspring at a given nest is potentially lower than for the females (Queller 1997; *Chapter II*; but see Houston & McNamara 2002).

Also, the number of potential mates may vary across populations, and likely increases with population size and density (Owens 2002; Forsgren et al. 2004; Kokko & Rankin 2006; McGraw et al. 2009). Assuming that population densities directly affect mating opportunities, this may have a pronounced effect on breeding behaviour of species with diverse breeding systems that involve alternative strategies. Mating opportunities appear to play an important role in determining the outcome of sexual conflict over care in species where one or both parents may desert (Székely et al. 1999; McNamara et al. 2000; Pilastro et al. 2001; Houston et al. 2005). As such, given that various strategies often have different reproductive payoffs mating opportunities should have a major impact on an individual's reproductive output (Smith & Sandell 2005; Szentirmai et al. 2007; Kokko & Jennions 2008; Maan & Taborsky 2008; Olson et al. 2008; *Chapter V*).

Theoretical models, field observations and comparative studies suggest that mating opportunities do influence sexual conflict over care. First, both dynamic optimization and game-theoretic models suggest that mating opportunity should influence parental care (Houston et al. 2005). For instance, improved mating opportunity for one sex induces more desertion (McNamara et al. 2000; Webb et al. 2002). Second, field studies show that the frequency of polyandrous females increases with the number of males available in several avian populations (Davies 1992; Pilastro et al. 2001). Third, a comparative study on birds suggested that families showing female-only care characterise species in which population densities are high and remating opportunities for both sexes are abundant, whereas those showing male-only care characterise species with low population densities and low frequency of mating opportunities (Owens 2002). One explanation for this pattern

may be that in internally fertilising species, such as birds, the males generally have the opportunity to desert earlier than females as the females still have to lay the eggs after copulation (Trivers 1972; Kokko & Jennions 2008). Males in a high density population will have the opportunity to find a new partner, whereas in a low density population it may be difficult for males to find a female which is in the right reproductive phase. Whether the above patterns stand within species has only rarely been studied before (Székely et al. 1999; Kosztolányi et al. 2006) and the relationship between ecological variables, breeding density and parental care is likely more complex than a mere association mediated through mating opportunities (Leisler et al. 2002; Kosztolányi et al. 2006; Alonzo & Sheldon 2009; Eldegard & Sonerud 2009).

The Eurasian penduline tit, *Remiz pendulinus*, has an unusually diverse breeding system among birds, in which sequential polygyny and polyandry regularly occur (Persson & Öhrström 1989; Szentirmai et al. 2007). Parental care is carried out by a single parent only, either by the male (at 5-20% of nests), or by the female (45-70%). The deserting parent abandons the clutch before incubation commences. Additionally, 30-40% of clutches is naturally deserted by both parents. The deserting parent may increase his/her reproductive success by remating while the full task of incubation and brood rearing is left to its mate (Persson & Öhrström 1989; Szentirmai et al. 2007). Biparental care is rare and it only occurs for a brief period during nestling feeding (Schroth & Helbig 1985; Franz 1989; Schönfeld 1989; Schönfeld 1994; O. Persson, pers. comm.). Biparental care has not been observed in a high-density population in Hungary (Szentirmai et al. 2007; *Chapter III*).

Here we test whether variability in breeding densities and mating opportunities are associated with different breeding strategies. We investigated the species' breeding system by comparing low density population (The Netherlands) with a high density population (Hungary). Firstly, we expected biparental desertion to be more frequent in a high density population, because the opportunity to find a new partner and thus enhance reproductive success will be better for both males and females, i.e. a higher incentive to desert. Similarly, given that biparental care does occur occasionally, as part of the species' breeding system we would expect this to be more common in low density populations with lower mating opportunities for males and females. In the latter case, biparental care may be a 'best out of a bad job'-strategy for both parents to stay and care for the offspring. Secondly, we predicted that high mating opportunities for females are associated with more female desertion.

Penduline tits are widespread throughout central and southern Europe (Burfield & van Bommel 2004); therefore we also investigated the plasticity of the breeding system by comparing the pattern of parental care strategies across five different populations.

## MATERIAL AND METHODS

We studied the breeding biology of Eurasian penduline tits in The Netherlands and in Hungary between 29 April 2006 and 1 August 2006. Penduline tits in The Netherlands were observed in six wetland areas (Foxhol: N 53°10'12.51", E 6°41'45.54"; De Groeve: N 53°17'16.75", E 6°50'13.82"; Veenhuizerstukken: N 53°00'43.02", E 6°59'00.89"; Kollummerwaard: N 53°19'32.90", E 6°11'44.07"; Rijnstrangen: N 51°53'07.03", E 6°02'25.37"; Ketelmeer: N 75 52°34'48.41", E 5°48'58.30"). These areas were separated by a minimum and maximum distance of 16.2 km and 78.9 km respectively. All six areas were similar in structure, containing various water bodies, reed beds and trees (mainly willow *Salix spp.* and poplar *Populus spp.*). In each of these areas plus surrounding areas nearby, we attempted to find all nests. Nests in both The Netherlands and in Hungary were repeatedly visited during nest building, pair formation, incubation and nestling feeding. Nests in The Netherlands were visited weekly, whereas in Hungary nests were visited at least every other day. Observations at the nest were performed using binoculars and/or a telescope for at least fifteen minutes per visit in Hungary and for at least one hour per visit in The Netherlands. Fifteen minutes are sufficient to record the presence of both parents (*Chapter III*). Nests in The Netherlands could not be visited as regularly as in Hungary for logistic reasons.

We collected data on breeding behaviour from 16 nests in the six areas in The Netherlands, and at 60 nests in Hungary, which were all studied at an extensive fishpond system, Fehértó (1321ha), in southern Hungary (see details in Bleeker et al. 2005). Fehértó consists of a number of ponds which are separated by dikes aligned by reed beds. Penduline tits used the trees on the dikes (mainly willow *Salix spp*., poplar *Populus spp.*, and Russian olive *Eleagnus angustifolius*) as nest sites and as a resource for nest building material and food (*Chapter VII*). Breeding in both the Dutch and the Hungarian populations was asynchronous, so potential new mates were available throughout the breeding season.

A parent was considered to have deserted the clutch if it was not seen at the nest for at least two consecutive visits (Szentirmai et al. 2005a). The type of parental

care (male-only, female-only, or biparental desertion) was determined for each pair that produced a clutch. In the Hungarian population birds were individually colour-ringed, whereas in The Netherlands birds were not ringed due to time deficiency. We attempted to make a distinction in The Netherlands between individual males and between individual females by taking digital photographs and using individual plumage characteristics (Kingma et al. 2008), supported by digital photographs. At fourteen out of sixteen nests we believe the males were different, whereas one male each built an additional nest. Females are difficult to distinguish individually, but we have no evidence that one female was involved in more than one attempt; although the latter seems unlikely, given the size of the populations in The Netherlands and distances between the areas. Several males and females were involved in more than one nest in Hungary (Pogány et al. 2008) although for the present study we randomly selected one nest for each individual male or female.

We estimated breeding density by counting the number of nests within a radius of 942m around each focal nest where a pair was present and egg-laying had taken place. We chose this cut-off point because this was the median distance between nests of individually marked females in southern Hungary (Mészáros et al. 2006). We anticipate that using a larger radius for the population in The Netherlands would produce a lower population density and lower mating opportunities, making our current results conservative. We performed a multinomial logistic regression to test if parental care types can be predicted based on breeding densities, where country was entered as a factor. Data included in this model provided an adequate fit (Pearson's goodness-of-fit: $\chi^2 = 30.71$, DF = 36, $p = 0.718$).

We estimated female mating opportunities for pairs of which date of pair formation and date of desertion were known. For these pairs we determined the number of unpaired males within a 942m radius around the nest during the period that the pair was together (i.e. the period from pair formation until desertion by one or both of the parents). We did not estimate mating opportunities for the males, because observations of unmated females are exceedingly rare. In addition, we compiled data on parental care in Germany, Sweden and Austria from published sources (Persson & Öhrström 1989; Franz 1991).

## RESULTS

Twenty-four and 158 nests were found in The Netherlands and Hungary, respectively, of which at 16 (67%) and 60 (38%) pair formation and egg-laying had

taken place, respectively. The proportion of nests where pair formation and egg-laying had taken place out of the total number of nests differed significantly between the two populations (Pearson $\chi^2 = 7.05$; DF = 1, $p < 0.001$).

Breeding density was significantly lower in The Netherlands (mean ± SD; 1.2 ± 0.5nests per km$^2$) than in Hungary (5.0 ± 2.1 nests per km$^2$; $U = 2.00$, $p < 0.001$, $N = 76$ nests), and the female mating opportunities were about ten times lower in The Netherlands (0.05 ± 0.12 unpaired males per km$^2$; Figure 8.1) than in Hungary (0.47 ± 0.37 unpaired males). However, the proportions of the three types of parental care, i.e. male-only care, female-only care and biparental desertion, were not different between The Netherlands and Hungary ($Z = 479.50$, $p = 0.994$, $N = 76$ nests; Figures 8.1 and 8.2) and they were unrelated to both breeding density (Table 8.1) and female mating opportunities ($U = 24.00$, $p = 0.442$, $N = 16$ nests; $U = 137.00$, $p = 0.258$, $N = 38$ nests, respectively).

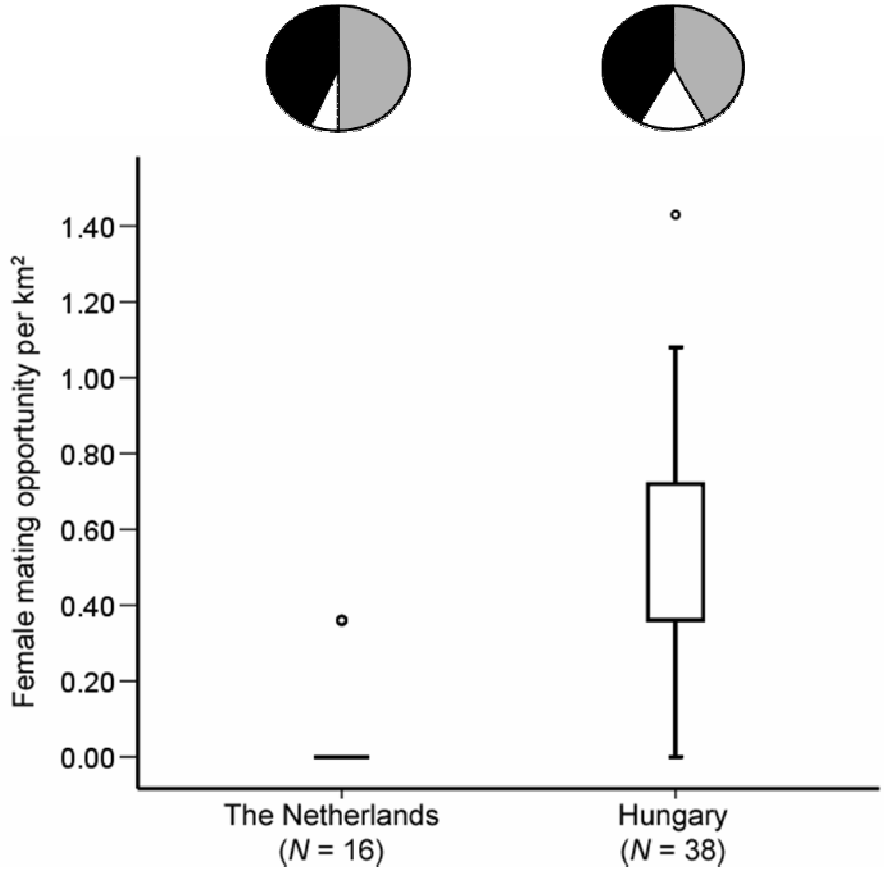


**Figure 8.1** Mating opportunities (number of unpaired males per 1 km$^2$ around each nest where pair formation and egg laying had taken place) are different between the penduline tit populations in The Netherlands and Hungary ($U = 94.00$, $p < 0.001$, $N = 54$). The plots indicate the interquartile range, the minimum and maximum and outliers are given by ○. The pie charts indicate the proportions of parental care in The Netherlands and Hungary ($N = 76$ nests). Shaded = female-only care, white = male-only care, and black = biparental desertion.

Incubation and feeding was performed by a single parent at all nests, and biparental care was not observed in either population. Consistently with these results, the patterns of parental care were not significantly different across five populations in Europe (Pearson $\chi^2 = 11.7$, DF = 8, $p = 0.16$, Figure 8.2).

## DISCUSSION

The resolution of sexual conflict over parental care in Eurasian penduline tits does not seem to be influenced by different breeding densities and mating opportunities. Our study clearly shows that within a single species there was no significant difference in parental care behaviour between the low-density (The Netherlands) and high-density (Hungary) populations, despite the fact that mating opportunities for females were significantly different between the two countries. Although some variation in pattern of parental care may be present, the overall breeding system of our study species is consistent across populations in Europe including the striking frequency of biparental desertion (some 30-40% in all populations), regardless of population density. This is in contrast with other studies where differences in type of parental care are explained by the number of available mates (Davies 1992; Balshine-Earn & Earn 1998; Székely et al. 1999; Pilastro et al. 2001; Owens 2002), which is presumably often directly associated with nesting density. We acknowledge that these results might be confounded by other factors or variables that vary across the populations included in this study, such as habitat quality, predation rates and climate. However, the consistency of the pattern of parental care across the five populations in Europe suggests that these variables may affect the decision to care or to desert to a minor extent only. We also note that the habitats in which the various populations occur are all very similar. All populations breed in reed marshes along lakes or rivers. Additionally, the variation in habitat structure within the Hungarian population of Eurasian penduline tits is not associated with the decision to care or desert (*Chapter VII*). With regards to mating opportunities, however, we note that we only included these for the females. If mating opportunities would have a similar effect on males as on females, i.e. males would have a similar reproductive advantage from remating as females, then the potential effect of mating opportunities on parental care may have been blurred in our study, since our predictions would not hold. However, this seems unlikely given the known sex difference in reproductive benefits from desertion in Eurasian penduline tits (Szentirmai et al. 2007; *Chapter V*).

**Table 8.1** Results of the multinomial logistic regression model of parental care strategy in response to breeding density and site (Hungary versus The Netherlands) in Eurasian penduline tits ($N$ = 76 nests, DF = 1). The reference category is biparental desertion. Predicted effect sizes and standard errors are given.

| Care strategy | | Model effect estimate (± SE) | Wald | $p$ |
|---|---|---|---|---|
| Female-only care | Site | 2.353 ± 1.912 | 1.515 | 0.218 |
| | Breeding density | -0.031 ± 0.137 | 0.051 | 0.821 |
| | Site*Breeding density | -2.098 ± 1.390 | 2.279 | 0.131 |
| Male-only care | Site | 0.920 ± 3.185 | 0.084 | 0.773 |
| | Breeding density | 0.000 ± 0.181 | 0.000 | 0.999 |
| | Site*Breeding density | -1.646 ± 2.509 | 0.430 | 0.512 |

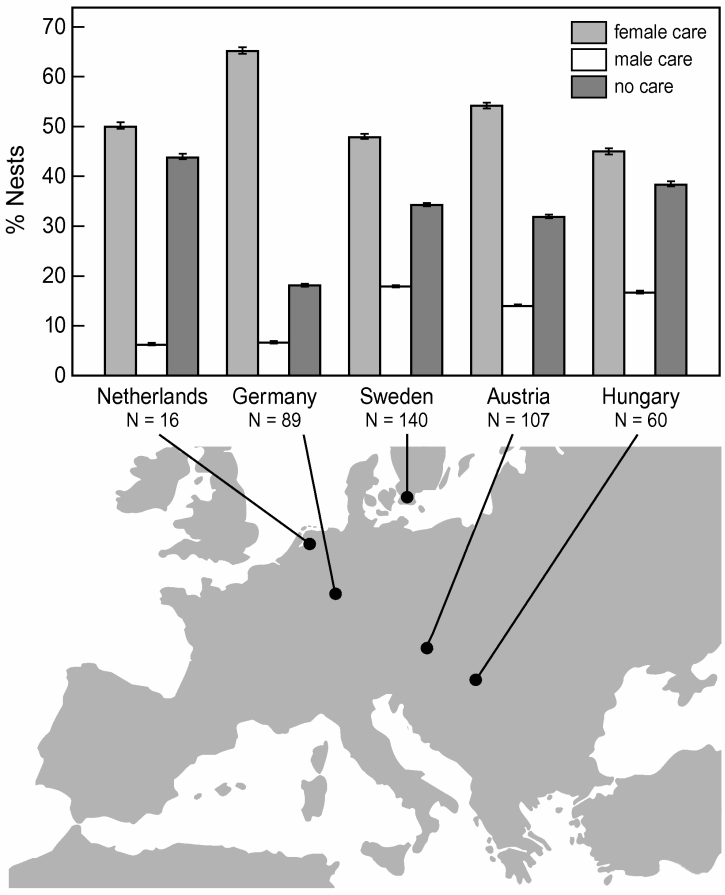


**Figure 8.2** Parental care in five populations of Eurasian penduline tits in Europe (Pearson $\chi^2$ = 11.7, DF = 8, $p$ = 0.16). The 'nests' on the y-axis refer to the nests at which pair formation and egg-laying had taken place (mean ± 95% confidence intervals; Germany: Franz, 1991; Austria: Franz, 1991; Sweden: Persson and Öhrström, 1989). Shaded = female-only care, white = male-only care, and black = biparental desertion.

Eurasian penduline tits show a clear seasonal pattern in parental care behaviour: there is more female care in the beginning of the breeding season, whereas males care more towards the end of the breeding season (Persson & Öhrström 1989; Szentirmai et al. 2005a). A decrease in mating opportunities for the male over the breeding season was suggested to be the most likely explanation for this seasonal pattern, with more females incubating or feeding offspring towards the end of the breeding season. Under that explanation mate availability would influence the resolution of sexual conflict over care in Eurasian penduline tits (Persson & Öhrström 1989). Our results do not support this view. However, the question whether these differences in mating opportunities may have affected the breeding system of Eurasian penduline tits on an evolutionary timescale remains unclear. Eurasian penduline tits started to inhabit The Netherlands only recently (van den Berg & Bosman 1999) and it may be that density dependent selection pressures have had no time to develop changes in breeding behaviour. It seems that patterns of parental care are fixed and this may suggest a genetic basis rather than a density dependent, environmental, effect on desertion behaviour. The species' migratory behaviour may also play an important role as to what extent local selective forces can influence the breeding system. High dispersion rates may lead to an increased gene flow and possibly annihilate local selection pressures (Mayr 1963; Garant et al. 2005; Price 2008). A study on consistency in desertion behaviour of Eurasian penduline tits showed that females were consistent in their desertion behaviour. Male desertion behaviour was found to be highly related to seasonality, and it was suggested that fixed genetic effects may be responsible for the decisions over care in females (Pogány et al. 2008). The latter, a fixed genetic effect, might also be true for the seasonality of a decision over care by males, rather than a direct effect of availability of potential mates.

The lack of support for the idea that the resolution of sexual conflict over care may depend on mating opportunities is also corroborated by the fact that we did not find any case of biparental care in the low-density populations in The Netherlands. Although we cannot entirely exclude the existence of biparental care, it at least seems extremely rare and as such hard to interpret as part of the species' breeding system.

Although we did not find any effect of breeding density on the Eurasian penduline tit's breeding system, in The Netherlands we did find a higher proportion of nests where pair formation and egg-laying had taken place compared to Hungary.

Thus penduline tits in The Netherlands appear more successful in pair formation and clutch production. This may indicate that birds are less choosy in a low density situation and males are less inclined to abandon their nest before they have attracted a partner. Thus mating opportunities may, to some extent, play a role in moulding the species' mating system.

Finally, from a conservation point of view, it is worth noting that with the inflexibility of the penduline tit's breeding system it is not unlikely that with decreasing numbers of potential new partners a decline in population size will suffer from a positive feedback loop: with a decreasing number of potential mates, the breeding success may rapidly decrease given that the benefits of desertion largely disappear (Szentirmai et al. 2007). This will potentially result in fewer available mates in future breeding seasons speeding up the process of a decline in numbers even further. In recent years the number of penduline tits appear to be declining across the edge of the distribution in Europe (Sweden: O. Persson, Italy: P. Tout, Germany: I. Todte, Spain: F. Valera, pers. comm.; The Netherlands: Netwerk Ecologische Monitoring SOVON, CBS, www.sovon.nl).

Future work may include experimental manipulation of breeding densities. We envisage this will be very difficult to carry out in the field, thus a captive population may reveal how breeding density or the availability of potential mates influences a decision to care or desert for the offspring. Furthermore, investigating genetic diversity among populations may provide a better understanding on dispersal patterns and levels of gene flow within this species. Radio-tracking of individuals may be a helpful tool to establish migration routes and dispersal patterns across populations. To what extent does gene flow take place between European breeding areas? Furthermore, investigating penduline tits in relatively isolated habitats found in, for instance, the Middle East and Kazakhstan where penduline tits have been present for much longer than in The Netherlands (Cramp et al. 1993), i.e. have had more time to evolve a more adaptive breeding system, may provide us with new information on the impact of mating opportunities on (1) the outcome of sexual conflict, or (2) the evolution of breeding systems within species.

**ACKNOWLEDGEMENTS**

We would like to thank all the fieldworkers that helped us collecting the data. We are grateful to the people who provided us with information on nesting locations in The Netherlands, in particular Martijn Bakker, Rommert Cazemier and Michel Geven. We are thankful to Dieter Franz (Germany) and Olof Persson (Sweden) for sharing their data on German/Austrian and Swedish populations. Ido

Pen and Michael Magrath provided us with valuable statistical advice, and Dick Visser kindly prepared figure 2 for us. Kiskunság National Park and Szegedfish Ltd. gave us the permissions to work at Fehértó (23864-2-3/2006). The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002-2006) under contract n. 28696. Further financial support came from grants to TS (Hungarian National Science Foundation OTKA (T043390), Royal Society Joint Project (15056), and the Leverhulme Trust (RF/2/RFG/2005/0279)), and from a University of Bath studentship to REvD.

# CHAPTER IX

# SEXUAL CONFLICT PREDICTS MORPHOLOGY AND BEHAVIOUR IN TWO SPECIES OF PENDULINE TITS

**René E. van Dijk, Ákos Pogány, Jan Komdeur, Penn Lloyd, and Tamás Székely**

*Manuscript*

*Authors' contributions*

**REvD**: study design, data collection, statistical analyses, manuscript preparation

**ÁP**: study design, data collection, manuscript improvement

**JK**: manuscript improvement

**PL**: data collection, manuscript improvement

**TS**: study design, manuscript improvement

**ABSTRACT**

The evolutionary interests of males and females rarely coincide over reproduction (sexual conflict), and these conflicting interests influence morphology, behaviour and speciation in various organisms. We examined the consequences of variation in sexual conflict in two closely-related passerine birds with contrasting breeding systems: the Eurasian penduline tit *Remiz pendulinus* (EPT) that has a highly polygamous breeding system with sexually antagonistic interests over parental care, and the socially monogamous Cape penduline tit *Anthoscopus minutus* (CPT). We derived four a priori predictions from sexual conflict theory, and tested these predictions using data collected in Central Europe (EPT) and South Africa (CPT). Firstly, we predicted that EPTs exhibit more sexually dimorphic plumage than CPTs due to more intense sexual selection. Secondly, we expected more attractive EPT males to provide less care than duller males. Thirdly, since song is a sexually selected trait in many birds, male EPTs were expected to exhibit more complex songs than CPT males. Finally, intense sexual conflict in EPT was expected to lead to low nest attendance as an indication of sexually antagonistic interests, whereas we expected more cooperation between parents in CPT. Consistent with our predictions EPTs exhibited greater sexual dimorphism in plumage and more complex song than CPTs, and more attractive EPT males provided less care than duller ones. Finally, EPT parents attended the nest less frequently and less simultaneously than CPT parents. These results provide support for sexual conflict theory, and are consistent with the notion that EPTs attempt to force their partner to work harder.

## INTRODUCTION

Reproduction has long been viewed as a cooperative exercise between male and female partners. Yet, the evolutionary interests of males and females are often different (sexual conflict, Parker 1979). Only in the rare case of semelparity, or when there is full and lifelong monogamy of the pair members will the optimum amount of care provided be equal for both parents (Lessells 2006; *Chapter II*). Only recently, however, have researchers started to explore the implications of sexual conflict on speciation, breeding systems, and evolution of various life-history traits (Gavrilets et al. 2001; Arnqvist & Rowe 2005; Hosken & Snook 2005; Houston et al. 2005). Sexual conflict is a potent evolutionary force that may mould morphology (Arnqvist & Rowe 2002a) and behaviour (Chapman et al. 2003), and promote speciation (Arnqvist et al. 2000). For instance, behavioural traits of dung fly *Sepsis cynipsea* populations undergoing more intense sexual conflict diverged to a greater extent than flies under more relaxed conflict, resulting in different levels of reproductive isolation (Martin & Hosken 2003). Extra-pair copulations in monogamous passerines may also result from sexually antagonistic evolution. The negative selection for direct benefits from extra-pair copulations for females appears to be greater than the positive selection for indirect benefits, which supports the notion that extra-pair copulations reflect pre-zygotic sexual conflict (Arnqvist & Kirkpatrick 2005).

Conflicts between parents over care (post-zygotic sexual conflict, Royle et al. 2002) emerge via a trade-off between parental effort and alternative mating opportunities for each parent. As a result, each parent may try to avoid the costs of care and shift those costs to its partner (Lessells 1999; Houston et al. 2005). This may happen through a continuous adjustment of parental effort in response to the mate's current effort (best response rule, Houston & Davies 1985), or through a discrete decision to either care for the offspring or to desert the partner and offspring (Maynard Smith 1977; Székely et al. 1996). Conflict over care typically occurs when there is an opportunity to reduce parental contribution. For example, a parent may desert the brood when one parent is sufficient to successfully raise the offspring (Bart & Tornes 1989; Székely et al. 1996). This may occur when resources are plentiful (Beissinger & Snyder 1987) or when offspring require little care, as is often the case with precocial young (Wisenden 1994; Olson et al. 2008). By deserting, the parent may benefit from finding a new mate and breeding again, thereby enhancing its reproductive success (Pilastro et al. 2001; Szentirmai et al. 2007; *Chapter II*; but see: Grüter & Taborsky 2005). As such, sexual conflict over care likely promotes

polygamous breeding (Davies 1989; McNamara et al. 2000; Magrath & Komdeur 2003; Székely et al. 2006), since with increasing levels of polygamy, variance in reproductive success increases. Thus, more polygamous breeding systems are associated with more intense sexual selection than monogamous systems (Björklund 1990; Wiklund & Forsberg 1991; Pérez-Barbería et al. 2002; Gonzalez-Voyer et al. 2008). Subsequently, sexual selection is expected to act stronger in species experiencing greater conflict.

Here we test a priori predictions of sexual conflict theory about the impact of sexual conflict on morphology and behaviour by comparing two closely related species of penduline tits (Gill et al. 2005; Alström et al. 2006): the sequentially polygynandrous Eurasian penduline tit *Remiz pendulinus* (henceforth EPT) and the socially monogamous Cape penduline tit *Anthoscopus minutus* (henceforth CPT). Our main objective is to elucidate the potential impact of sexual conflict on the evolution of traits, which may eventually facilitate speciation (van Dijk et al. unpubl. data).

The EPT is a small passerine (body mass about 9g) with a widespread distribution across Europe and Central Asia. Intense conflict between parents is indicated by several studies that showed that parental care is carried out by a single parent only, and both the male and the female endeavour to desert before the other (Persson & Öhrström 1989; Szentirmai et al. 2007; *Chapter III*). In addition, about one third of clutches is deserted naturally by both parents; a pattern consistent between five European populations (Persson & Öhrström 1989; Franz 1991; Schleicher et al. 1997; Szentirmai et al. 2007; *Chapter VIII*). Both polygyny and polyandry are common, since the deserted parents often obtain new mates, so that both sexes may mate with up to six partners in a single breeding season. By deserting the clutch both males and females enhance their own reproductive success, whereas caring reduces reproductive success in both sexes (Szentirmai et al. 2007; Fig. 9.1). In contrast, the CPT (body mass about 6g, endemic to southern Africa) is socially monogamous, and parents cooperate to incubate the eggs and rear the brood together, sometimes assisted by helpers at the nest (Harrap & Quinn 1996; Dean 2005). The pair usually stays together throughout and sometimes across breeding seasons (Lloyd P, van Dijk RE, Pogány Á unpubl. data).

Firstly, given that EPT is frequently polygamous and thus likely experiences a larger variance in reproductive success, we predicted more intense sexual selection

in EPT than in the socially monogamous CPT. This is expected to result in a stronger sexual plumage dimorphism and more complex song in EPT than in CPT.

Secondly, we tested the prediction that male EPTs with a large eye-stripe, which signals male attractiveness (Pogány & Székely 2007; Kingma et al. 2008), desert the nest at a higher frequency than less attractive males, thus imposing the costs of parental care on their mate. We expected attractive males to desert more frequently than less attractive males, since attractive males likely procure new mates after desertion. As such, more attractive males should derive greater benefits from desertion to offset the potential cost of biparental desertion. Females mated to more attractive males, however, face the costs of care and/or reduced reproductive success (Szentirmai et al. 2007; Fig. 9.1).

Finally, following predictions from sexual conflict theory (e.g. Houston et al. 2005), we expected that in EPT, in which nest desertion is common, parents will attempt shifting the costs of care to their mate. Specifically, we predicted EPT pairs to attend the nest less frequently and less synchronously during the egg-laying phase than in CPT. Given the intense conflict in EPT, a parent may abstain from building a nest expecting its mate to make up the shortfall. In the cooperating CPT we predicted synchronous nest attendance and nest building by both parents. Nests of both species are sophisticated structures (see below) and built by both sexes.

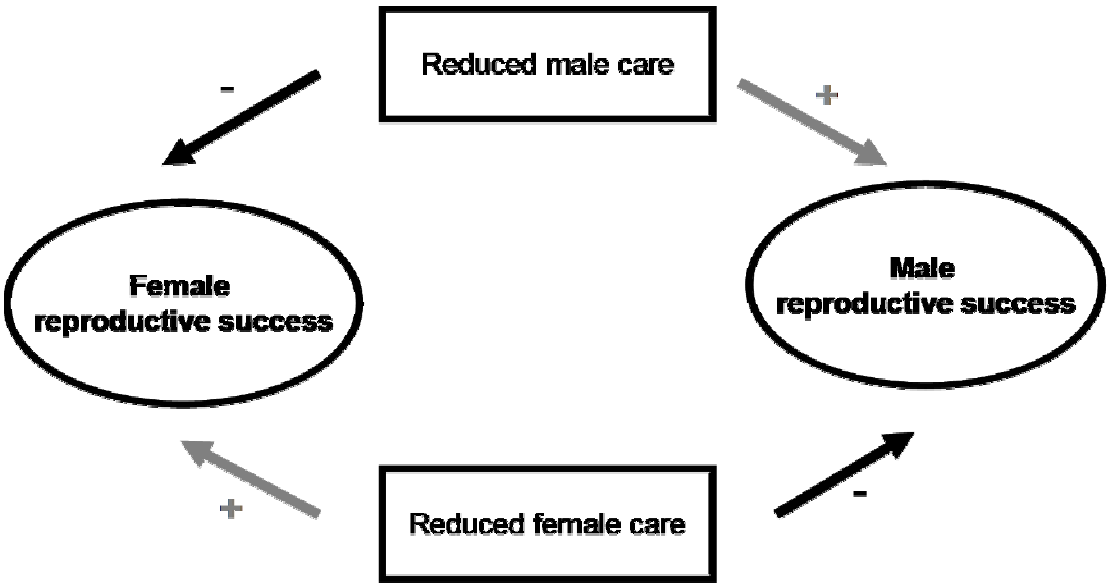


**Figure 9.1** Sexual conflict in Eurasian penduline tits (after Szentirmai et al. 2007)

## METHODS

*Study sites and data collection*

We studied EPTs between April and August in five consecutive breeding seasons (2003-2007) in a reed marsh at a 1321ha fishpond system, Fehértó, in southern Hungary (46º19'N 20º6'E), where approximately 60-90 males and 45-50 females bred each year. We studied eight and six breeding pairs of CPT in September 2006 and 2007, respectively, in coastal scrubland at the 572ha Koeberg Nature Reserve near Cape Town, South Africa (33º40'S 18º26'E). The low number of monitored nests in CPT compared to EPT is due to the lower population density in CPT, as large territories are used by family groups (Dean 2005). Both species build similar, domed nests, initiated by the male. In EPT males are unpaired, whereas most CPT males are paired at the onset of building. The nest is finished and maintained jointly by both male and female after pair formation in both species. The egg-laying phase is initiated at a similar stage of nest building, i.e. when the parents start building the entrance tube to the nest.

We searched both study areas for nest-building penduline tits, and visited each nest about every other day to determine which parent attended the nest (*Chapter III*). At each EPT nest we recorded the date of pair formation. We considered a male to be mated as soon as the pair was seen copulating near the nest or when male and female were seen to build the nest together. For time in season we used a date format as the number of days since 1 April in each year. We trapped and banded birds with one numbered metal band from the Hungarian Ornithological Institute (EPT) or the South African Bird Ringing Scheme (CPT), and a unique combination of three color bands (A.C. Hughes, Middlesex, UK). Three digital photographs were taken of each side of the bird's head using an Olympus FE-100 and a Fujifilm FinePix A203 digital camera. In all photographs we kept a ruler in the background as a reference to measure the size of the eye-stripes. The birds were hand held touching the ground and the camera was positioned at an approximately fixed distance (about 20cm) from the bird to standardise aberrations. The area of the eye-stripe (to the nearest 0.01 $cm^2$), signaling attractiveness in EPT (Pogány & Székely 2007; Kingma et al. 2008), was quantified from the digital photographs using Adobe Photoshop 7.0. We took the average of the three measurements for the size of the eye-stripe.

The song of 16 male EPTs was recorded in 2006 for 127.5 ± 48.4min (mean ± SD) at a randomly selected time of day between 06:28 and 17:50 (CET), using a Marantz PMD 660 portable digital recorder with a Sennheiser ME66 directional

microphone. Using the same equipment as for EPT, we recorded the song of 9 CPT males (recording time 220.9min ± 94.3min). All recordings for CPT were made during the morning (06:20 – 11:30 UTC). For both species, in the analyses we only included song recordings from mated males. Sonograms of the recordings were created and analyzed using Audacity v. 1.2.6 and Avisoft-SASLab Light v. 3.74.

To investigate nest attendance during nest building, which continues through the laying period, we filmed nests in 2006 and 2007 in both EPT and CPT using a time-lapse video camera (Sony digital handycam, DCR-HC44E) storing one frame every five seconds. In CPT we knew precisely the date when the first egg was laid, and nest attendance of parents was recorded during the second and third days of egg-laying (547min ± 82min per day, $N$ = 7 pairs). In EPT egg-laying dates were often not known, therefore we recorded nest attendance from after pair formation and during egg-laying for EPT (329min ± 184min per day, $N$ = 21 pairs), i.e. a more extended period than for CPT. The period before egg-laying involves more nest building than maintenance. We anticipate that this would not influence our results, since the parents are expected to spend more time at the nest during nest building than during nest maintenance, which would result in more nest attendance in EPT than in CPT. The pattern we predicted and found is opposite to this (see Results). Recordings were analyzed frame by frame using MATLAB v. 6.5 (256240 and 96632 frames in total for EPT and CPT, respectively), coding nest attendance (i.e. presence of bird on or inside the nest) as: (i) male-only, (ii) female-only, (iii) joint nest attendance by male and female, or (iv) both parents absent.

To distinguish male and female parents from intruders we used individual differences in plumage (Cramp et al. 1993; Kingma et al. 2008), behaviour (e.g. females are more often and for longer periods inside the nest than males; intruders are often on the outside of the nest and build very little), and color bands. Ambivalent records, i.e. when the identity of an individual was ambiguous, were excluded (7.1% and 4.8% of total records of EPT and CPT, respectively). As nest desertion takes place during egg-laying in EPT, we only included pre-desertion records.

*Data analyses*

We used binary logistic regression models with backward elimination to predict parental care strategy (male or female as response variable; care/desert) at the first clutch of EPT in response to the size of the male eye-stripes. The initial model

included year as a categorical covariate and mating date as a continuous covariate. Neither covariate contributed significantly to the model ($P > 0.255$), so both were removed from the final model. The final model provided an adequate fit to both male strategy (Hosmer-Lemeshow goodness-of-fit; $\chi^2 = 10.289$, df = 8, $P = 0.245$), and female strategy ($\chi^2 = 6.400$, df = 8, $P = 0.603$).

All CPTs videotaped were color banded. The analyses for nest attendance by CPTs included one male that was recorded at two nests in consecutive years with a different female, so we included these as two data. Out of the 21 EPT nests filmed, one male and 18 females were not color banded. Adult returning rates between years are low (5% for males, 2% for females; *Chapter VII*), therefore it is unlikely that we observed the same unbanded individuals in different years. Additionally, of eight unbanded females in 2006 and the ten in 2007, three and six bred simultaneously, respectively, and we can thus be certain that these are different individuals. For the remaining nine females we cannot exclude the possibility of pseudoreplication, although we suspect it is unlikely given (i) the size of our breeding population and (ii) that the composition of pairs was nearly always different (out of 194 pairs that produced a clutch, only six pairs remained together and produced a second clutch between 2002 and 2007). Pseudoreplication in the plumage analyses was avoided by randomly choosing one measurement per individual.

To examine the degree of synchrony in nest attendance by male and female, i.e. male and female being together at the nest simultaneously, we first calculated the time that the male and female can be expected to spend together at the nest by chance, by multiplying the total percentage nest attendance by the male, i.e. male-only attendance plus attendance by male and female together, with the total percentage nest attendance by the female. We then compared the difference between observed and expected patterns of nest attendance by both species using a General Linear Model (GLM). A GLM was also used to compare the total proportion of time the parents spent at the nest, i.e. the sum of male-only, female-only and joint nest attendance, between the two species. Both GLMs included year as a factor and the first day of filming as a covariate, although neither contributed significantly to either of the models ($P > 0.138$) so they were excluded from the final models. Day lengths are different between Hungary (15 h 46 min ± 0 h 11 min, Budapest) and South Africa (11 h 41 min ± 0 h 12 min, Cape Town) and to test whether this might confound our results we estimated the absolute time the parents attended the nest per day as the percentage of time spent at the nest x day length (day lengths for both

study sites collected from http://www.timeanddate.com). We then compared whether the absolute time spent at the nest by both parents is different between EPT and CPT.

We provide effect sizes (Cohen 1988) and power analyses, and applied the asymptotic relative efficiency when estimating power of Mann-Whitney U-tests (Lehmann 1975). If the power of the statistics was relatively low for CPT (i.e. $1\text{-}\beta \leq 0.5$), we provide the sample size that would be required to find a statistical significant difference between the two groups given Cohen's effect size $d$ of the underlying data of CPT and power $1\text{-}\beta = 0.8$ ($N_{required}$), and the required sample size given the effect size $d$ in EPT and power $1\text{-}\beta = 0.8$ ($N_{d,1\text{-}\beta}$). All statistical analyses were performed using SPSS 14.0.0 (SPSS Inc., USA), except power analyses, which were carried out in R (R Development Core Team 2005). We provide mean ± SD, and two-tailed probabilities.

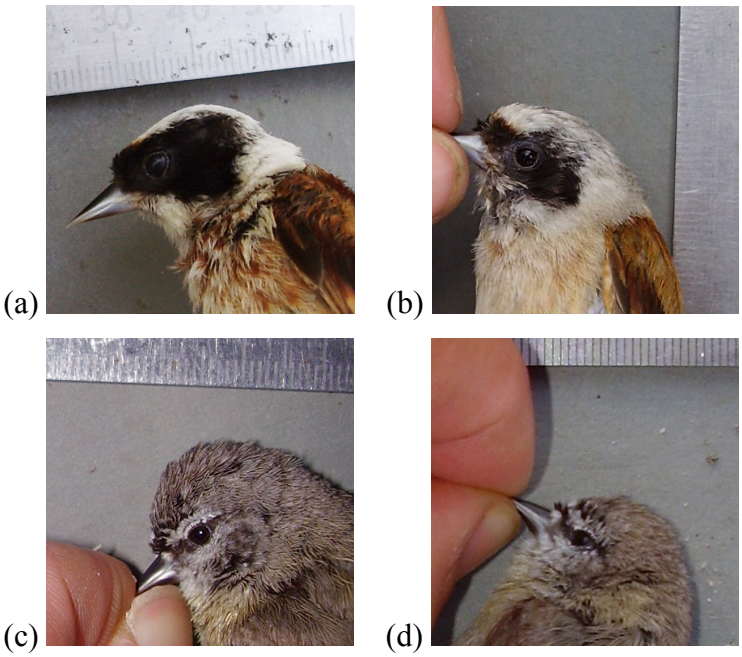


**Figure 9.2** The size of the eye-stripe of (a) male and (b) female Eurasian penduline tits, and (c) male and (d) female Cape penduline tits.

## RESULTS

### *Plumage*

In EPT, the eye-stripe of males was significantly (28%) larger than that of females, whereas in CPT the size of the eye-stripe was not different between males and females (Fig. 2; sex: $F = 9.881$, $P = 0.002$; species: $F = 295.358$, $P < 0.001$; interaction sex x species: $F = 10.290$, $P = 0.002$, $N = 206$ individuals; Table 9.1). Male EPTs with large eye-stripes deserted their first clutch more often than those

**Table 9.1** Mask size of male and female penduline tits. *d* = Cohen's effect size, 1-β = power. The sample size required for a statistically significant difference is provided for CPT given the effect size *d* of the underlying data of CPT and the power 1-β set at 0.8 ($N_{required}$), and given the effect size *d* in EPT and the power 1-β set at 0.80 ($N_{d,1-\beta}$) (see Cohen 1988).

| | Males ($cm^2$) | Females ($cm^2$) | | *P* | *d* | 1-β | $N_{required}$ | $N_{d,1-\beta}$ |
|---|---|---|---|---|---|---|---|---|
| EPT | 1.29±0.23 (*N* = 155) | 0.93±0.20 (*N* = 34) | *t* = 8.419 | <0.001 | 1.594 | > 0.99 | | |
| CPT | 0.13±0.02 (*N* = 9) | 0.14±0.03 (*N* = 8) | *Z* = 0.627 | 0.531 | 0.034 | 0.05 | 13581 | 8 |

with small and thus less attractive eye-stripes (Fig. 9.3; binary logistic regression model; model effect estimate ± SE = 2.647 ± 1.226, Wald = 4.661, *df* = 1, *P* = 0.031, *N* = 121 males). Females, however, did not care more often for clutches of males with large eye-stripes (0.222 ± 0.786, Wald = 0.080, *df* = 1, *P* = 0.778, *N* = 121 males).

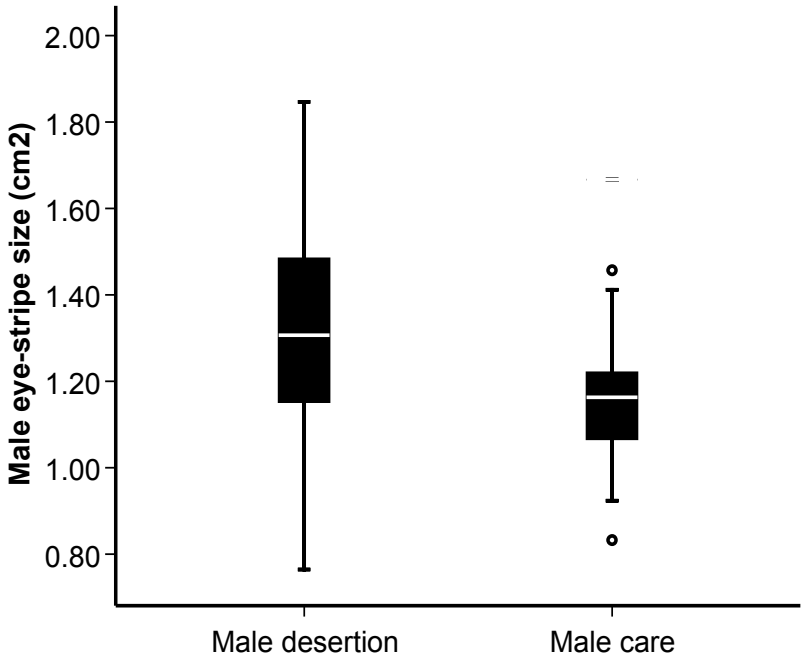


**Figure 9.3** Eye-stripes of deserting male Eurasian penduline tits (*N* = 104) are larger than the caring ones (*N* = 17). Boxplots show median, interquartile range, outliers and extreme cases.

*Song*

From the song recordings of EPT a total of 2229 syllables was analyzed. Adding all recordings from all males together, the total number of different syllables sung in the

population ('repertoire size') in EPT did not increase after 46% of the total time of recordings (2100min). Additionally, after the first 52% of recorded syllables (i.e. the first hour of recording from all 16 males) we obtained 14 out of the 16 different syllables we recorded in total (i.e. 88%). All different syllables sung by an individual male were obtained after 71% ± 24% of the total number of syllables recorded per individual. We may have underestimated the repertoire size for individual males, although this would only make our results more conservative (see below). The song recorded from CPT contained a total 1918 syllables. We did not find variation in the number of different syllables sung by CPT, so we are confident that we obtained the full repertoire size for CPT. The song output at the nest was not significantly different between species: EPT males sang 62.8 ± 32.0 ($N$ = 16 males) syllables per hour, whereas CPT males sang 54.0 ± 42.8 ($N$ = 9 males) syllables per hour ($t$ = 0.581, $P$ = 0.567, $N$ = 25 males, $d$ = 1.461, 1-β = 0.92). EPTs used 8.3±2.8 different syllables ($N$ = 16 males), whereas song was invariably mono-syllabic in CPT (Fig. 9.4; one-sample $t$-test with test value = 1; $t$ = 10.474, $P$ < 0.001, $d$ = 3.029, 1-β > 0.99).

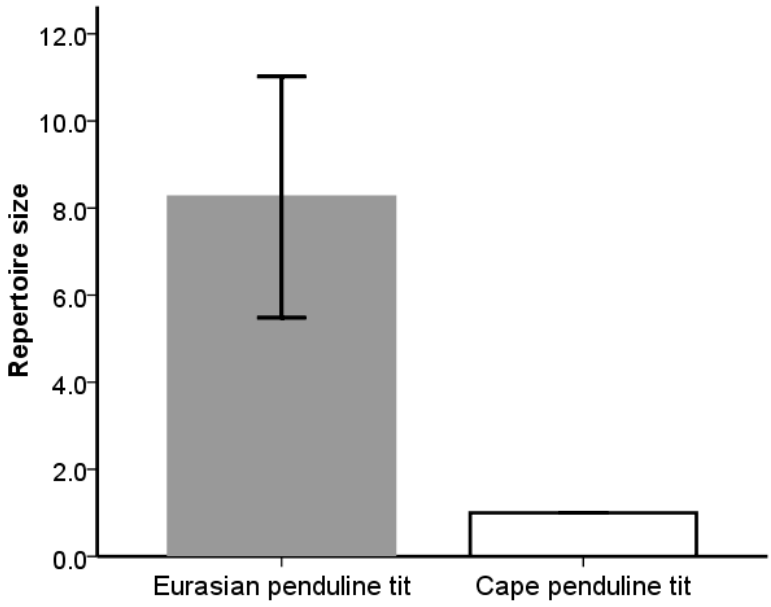


**Figure 9.4** Repertoire size, i.e. the mean number of different syllables sung by each male, in Eurasian and Cape penduline tit. Bars represent mean ± SD.

*Nest attendance*

Eurasian and Cape penduline tits differed significantly in the frequency of synchronous nest attendance by male and female (Fig. 9.5; Mann Whitney U; $Z$ = ±

3.902, $P < 0.001$, $N = 28$ pairs, $d = 2.949$, 1-β >0.99). This result was corroborated by comparing the absolute time spent at the nest by male and female jointly (EPT: 115s ± 162s, CPT: 2343s ± 864s; Mann Whitney U; $P < 0.001$).

Comparing the expected versus observed times at the nest by both parents, we found a significant effect of species ($F = 20.366$, $P < 0.001$, $N = 28$, $\eta^2 = 0.439$, 1-β = 0.99): EPT parents spent significantly less time together at the nest than expected by chance (Fig. 9.5; 0.20% versus 3.23% of time, respectively; $Z = \pm 4.015$, $P < 0.001$, $N = 21$ EPT pairs, $d = 2.898$, 1-β > 0.99), whereas in CPT the expected *versus* observed times were not different (Fig. 9.5; 5.56% versus 5.05% of time, respectively; $Z = \pm 0.845$, $P = 0.398$, $N = 7$ CPT pairs, $d = 0.329$, 1-β = 0.09, $N_{required} = 146$, $N_{d,1-\beta} = 4$).

EPTs spent significantly less time at the nest (36.0 ± 9.9%, $N = 21$ pairs) than CPTs (49.4 ± 15.7%, $N = 7$ pairs; $F = 7.075$, $P = 0.013$, $\eta^2 = 0.214$, 1-β = 0.726).

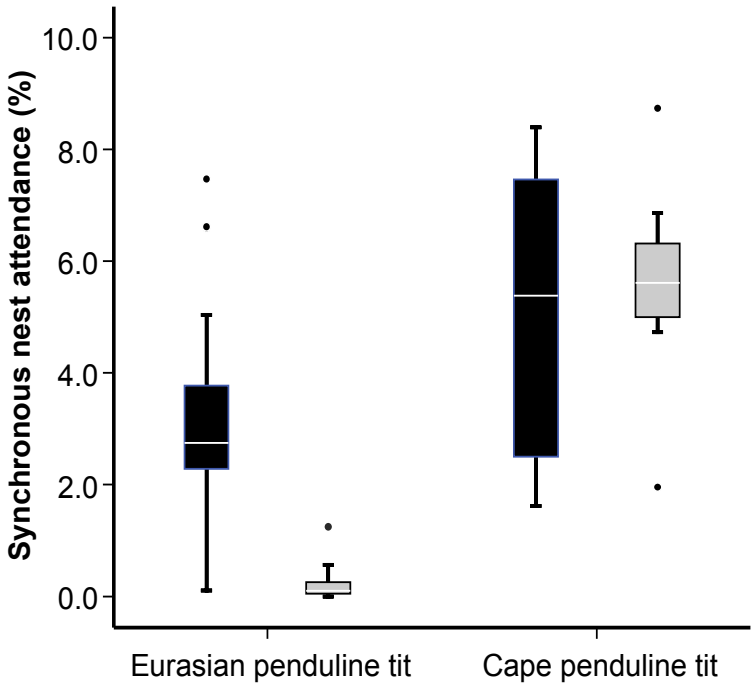


**Figure 9.5** Expected (black boxes) and observed (shaded boxes) synchronous nest attendance by male and female Eurasian and Cape penduline tits. Boxplots show median, interquartile range, outliers and extreme cases.

## DISCUSSION

Penduline tits (Remizinae) are emerging as one of the model systems in investigations of parental conflict (Persson & Öhrström 1989; Arnqvist & Rowe 2005; Szentirmai et al. 2007; McGraw et al. 2009; *Chapter II*) and here we found

support for several a priori predictions flowing from sexual conflict theory. Firstly, we found substantial sexual plumage dimorphism in EPT, but not in CPT. Secondly, we found that EPT males had a more complex song (i.e. a larger song repertoire) than CPT males. These results together suggest that sexual conflict may drive the evolution of plumage dimorphism and complexity of song through intensified sexual selection. We realise that the power to detect a significant difference between the sexes of CPT in size of the eye-stripe is low, and we acknowledge that this may have confounded this result. However, the sample size required to detect a sexual dimorphism in the size of the eye-stripe in CPT, given the effect size and power ($N_{\text{required}}$), is unrealistically large (13581), yet with our sample we would have been able to detect a sex difference in eye-stripe size in CPT if it had been of a similar intensity as in EPT ($N_{d,1\text{-}\beta}$; Table 9.1). Therefore male and female CPTs appear factually monomorphic in the size of the eye-stripe. Additionally, we note that males and females can easily be distinguished in the field in EPT, but not in CPT (see various field guides), and we thus suspect our results would be robust even with much larger samples sizes.

Thirdly, we found that EPT females mated to attractive males were more likely to be deserted by their partner. However, these females do not care more often for the offspring after male desertion than females mated to less attractive males. The latter could be interpreted as retaliation by the females to avoid being exploited by the males, and may partly explain the existence of biparental desertion: if an attractive male deserts, a female may still desert, despite the loss of the eggs (see also *Chapter III*). This somehow contradicts the prediction of the differential allocation hypothesis (Burley 1986; Sheldon 2000). Nevertheless, females mated to attractive males pay additional costs of reproduction compared to females mated to less attractive males: a female may obtain both direct and indirect benefits from attractive males, yet these females pay the full costs of caring or, in case they desert too, their efforts invested in nest building and egg-laying, appear to be in vain. In addition, indirect benefits are generally assumed not to offset the direct male imposed costs, resulting in indirect benefits being of little importance in the evolution of sexually antagonistic traits (Cameron et al. 2003; Arnqvist & Kirkpatrick 2005; Chapman 2006). Thus, the fitness of a female is probably reduced when mated to an attractive male, consistent with the prediction of sexually antagonistic coevolution (Chapman et al. 2003; Szentirmai et al. 2007). This points to the dilemma of EPT females: by choosing an attractive mate she may actually lose (Chapman et al. 2003).

Our prediction that the intensity of sexual selection increases with more polygamous breeding systems (Björklund 1990; Andersson 1994; Székely et al. 2007) was supported by our results with regards to the differences in plumage dimorphism and song complexity, but is inconsistent with our result that females pay a cost of mating to an attractive male relative to mating with a less attractive male. Our results, in addition to Kingma et al. (2008) showing that female reproductive success (number of nestlings and their survival) tend to decrease with their mate's attractiveness, suggest that males may manipulate their partner via sexually selected traits.

Sexual conflict may be associated to a process of manipulation by one partner and resistance by the other. This potentially affects the evolution of various traits (Chapman et al. 2003; Lessells 2006), and may also explain the difference in sexual dimorphism and song complexity between the two species of penduline tit. Evidence for this arms race between male and female partners derives from pre-copulatory sexual conflict where males are harmful to females during copulation (Crudgington & Siva-Jothy 2000; Arnqvist & Rowe 2002a; Arnqvist & Rowe 2002b; Lessells 2006). Males may, for instance, cause genital damage to the female (Crudgington & Siva-Jothy 2000), or force the female to mate at a suboptimal rate (Arnqvist & Nilsson 2000; Arnqvist & Rowe 2002a). There is good evidence demonstrating that females may pay substantial fitness costs of mating, such as reduced longevity and/or offspring production (Gavrilets et al. 2001; Arnqvist & Rowe 2005; Fiumera et al. 2006). Males may also try to exploit the female's perception system during mate choice and parental investment. Exploitation of females may be most successful through exaggerated sexually selected traits in males, such as ornaments or song. Females are expected to counter-adapt through more selective mate choice (Gavrilets et al. 2001; Chapman et al. 2003; Arnqvist & Rowe 2005) leading to female resistance to mating and the evolution of exaggerated male display to overcome this resistance ('sexually antagonistic coevolution', Dawkins & Krebs 1978; Holland & Rice 1998; Chapman et al. 2003). In that light, the evolutionary driving force of preference is resistance to male-imposed costs, rather than gaining benefits from mating with preferred males, as described under classic sexual selection (Holland & Rice 1998; Gavrilets et al. 2001; Chapman et al. 2003). Mediated by this dynamic process of manipulation and resistance, sexual conflict may have a pronounced influence on the evolution of both morphological and behavioural traits: Male EPTs may try to manipulate their partner via elaborate plumage and song. This, in

conjunction with resistance by the female, may have led to the exaggeration of those traits in EPT, but not in CPT.

An alternative explanation for the elaboration of traits in EPT may be the higher population density than in CPT. Several authors have suggested that higher population density may promote greater sexual selection by increasing competition for mates (e.g. Kvarnemo & Ahnesjö 1996; Owens 2002), but the generality of this mechanism has been challenged (Kokko & Rankin 2006; Head et al. 2008).

Breeding density itself is not a selective process, but rather an environmental trait that amplifies or de-amplifies sexual conflict. To separate the potential of breeding density from sexual conflict, one needs to compare different populations of the same species at different breeding densities and investigate local adaptations.

Finally, we found that the extent of cooperation in the breeding system was reflected in parental behaviour, quantified as the time spent on nest attendance. As expected, we found that EPT parents not only spend less time overall on nest attendance than CPT, but they also were at the nest less synchronously. The latter was also true when we compared the estimated absolute time the parents spent jointly at the nest, confirming that the difference in day length between our two study sites does not alter our results. These results suggest that EPT parents appear to avoid each other at the nest, in order to force the partner to do the work. The energy saved may then be invested in a next reproductive bout in EPT, whereas in CPT, where there are fewer opportunities for future reproduction, parents would benefit from more intense mutual cooperation at a given nest. An alternative explanation for the observed pattern in EPT is role division so that EPT partners may take over the job of nest building from each other rather than actively avoiding each other at the nest. This would also result in less time spent together at the nest, albeit that this should be interpreted as a more cooperative behaviour as opposed to avoidance due to conflict. The fact that the total time spent on nest attendance (total frequency of individual and joint attendance) is lower in EPT than in CPT, however, corroborates the idea that they actively try to avoid each other, rather than taking over each other's work as a cooperative effort (see Royle et al. 2002).

Furthermore, the mate guarding hypothesis (Kempenaers et al. 1995; Birkhead 1998; Møller & Ninni 1998) is unlikely to explain the difference in joint nest attendance between the two species: due to frequent mate change and dense breeding population in EPT, one would predict more intense mate guarding in EPT than in CPT. However, we found the opposite pattern.

To conclude, our results are consistent with the predictions from sexual conflict theory, and suggest sexual conflict may influence the evolution of morphological and behavioural traits in penduline tits. Nevertheless, we also acknowledge alternative selective processes that may influence these traits by acting themselves or acting with sexual conflict. To establish the generality of these results and to test alternative hypotheses, we need phylogenetic comparative studies using the appropriate framework. We are currently working on the first comprehensive phylogenetic hypotheses for Remizinae (van Dijk et al, in prep.), which will serve as backbone for future analyses (Harvey & Pagel 1991; Freckleton et al. 2002; Thomas & Székely 2005). Detailed data from the field, collected from multiple, closely-related species exhibiting a variety of breeding systems in various habitats will further advance this field. The diverse breeding systems of penduline tits are therefore an excellent model system to understand how sexual conflict and cooperation may have shaped the morphology, behaviour, ecology and evolution of organisms.

**ACKNOWLEDGEMENTS**

The work was supported by the European Community's Sixth Framework Programme [FP6/2002-2006] under contract n. 28696; a University of Bath studentship to REvD; the Schure-Beijerinck-Poppingfonds of the Royal Netherlands Academy of Sciences [SBP/JK/2007-53 to REvD]; the Hungarian-South African Intergovernmental TÉT [OMFB-00564/2006 to TS, AP, and PL]; the Hungarian National Science Foundation OTKA [T043390 to TS]; a Royal Society Joint Project grant [15056 to TS]; and the Leverhulme Trust [RF/2/RFG/2005/0279 to TS]. We are grateful to all people who helped with the fieldwork in Hungary over the years. We thank the Kiskunság National Park, Szegedfish Ltd. (Hungary), and ESKOM (South Africa) for permission to carry out fieldwork at the field sites. We also thank Johan van der Dennen for writing the MATLAB script to analyze the time-lapse video recordings, and Sander Bot for analyzing EPT song.

# CHAPTER X

## CONCLUSIONS AND FUTURE DIRECTIONS
## SEXUAL CONFLICT OVER CARE IN PENDULINE TITS AND BEYOND

**René E. van Dijk**

Should a parent care for its offspring, or should it leave the care provisioning to its mate? This question lies in some of the core themes in sociobiology, behavioural ecology and human social behaviour. The answer, as I argue in this thesis, depends on a suite of social and non-social variables. I show how sexual conflict over care may be influenced by the behaviour of the partner, the attractiveness of the mate, and the environment (*Chapters III – VIII*). I evaluate these results predominantly in a temporal, ecological context, i.e. how do these variables influence a decision over parental care in a given breeding season. However, I argue that environmental variables have also played a role in an evolutionary context, i.e. they may have influenced the evolution of the penduline tits' diverse breeding systems. In *Chapter IX* I present some of the potential evolutionary consequences on behavioural and morphological traits of a breeding system with intense sexual conflict over care.

In this final chapter I focus on five major results of my thesis (see Table 10.1):

i. the process of clutch desertion is rapid in Eurasian penduline tits (*Chapters III and IV*);
ii. parents appear to conceal - and not behaviourally signal - their intention to desert the clutch (*Chapters III and IV*);
iii. the pattern of parental care strategies is consistent with predictions based on reproductive payoffs using a game-theoretic analysis (*Chapter V*);
iv. parental care strategies of Eurasian penduline tits are not confounded by sex differences in parental quality (*Chapter VI*), and are unrelated to either habitat structure (*Chapter VII*) or breeding density (*Chapter VIII*);
v. sexual conflict appears to have important ramifications on behavioural and morphological traits in Eurasian and Cape penduline tits (*Chapter IX*).

# 1. The process of clutch desertion

*How does a decision about parental care depend on the partner?* Various behavioural and morphological traits are expected to influence social interaction of parents. Here I focus on a dichotomous decision: care for the offspring or desert. In this social context, a number of trade-offs is expected to play a role, such as:

- should a parent 'plan ahead' and save resources that can be invested in future reproduction if he/she is going to desert, or should the concealment of the intention to desert be favoured?

**Table 10.1** Main conclusions of my thesis work in regards to the two major questions of *Chapter I*.

| *Chapter* | *How do parents decide over parental care?* | *The evolutionary ramifications of sexual conflict* |
|---|---|---|
| III, IV | - nest desertion is a rapid process in Eurasian penduline tits<br>- nest desertion depends on the behaviour of the partner<br>- males desert either in early morning or late afternoon, whereas females desert any time of the day | - parents do not signal their intention to desert |
| V | - parents are not caught in the Prisoner's Dilemma, but play the coordination game<br>- a large part of variation in parental care is based on reproductive payoffs | - both sexes benefit from desertion, rather than providing parental care, in terms of reproductive payoffs |
| VI | - males provide as effective parental care as females | - males care for smaller clutches than females in Eurasian penduline tits |
| VII | - the structure of habitat does not predict parental care | |
| VIII | - patterns of parental care are not different between high-density and low-density populations (Hungary versus The Netherlands) | |
| IX | - more attractive males are likely to desert the clutch<br>- females mated to attractive males do not provide more care than females mated to non-attractive males | - breeding systems with intense sexual conflict are associated with larger sexual dimorphism in plumage than in a cooperative systems |

| *Chapter* | *How do parents decide over parental care?* | *The evolutionary ramifications of sexual conflict* |
|---|---|---|
| IX | | - intense sexual conflict is associated with a complex song repertoire<br>- penduline tits exhibiting intense sexual conflict attempt to shift the costs of parental care to the partner, whereas cooperative species do not |

- should a female mated with an attractive male put up with the risk of being deserted, or would she be better off with a less attractive male that may rear the offspring?

I envisage that a process of manipulation by one parent, and the subsequent resistance by the other parent, may lead to an evolutionary cycle (Chapman et al. 2003): males may develop attractive traits to manipulate the female into providing more care for the offspring. This is beneficial for the male, but will reduce fitness of the female given that these males unlikely to care for her young (Arnqvist & Rowe 2005; Fiumera et al. 2006). Females then are expected to overcome this manipulation by being more selective among males and resisting the courtships of attractive males (Gavrilets et al. 2001; Chapman et al. 2003; Arnqvist & Rowe 2005; Fig. 10.1).

In my thesis, I have shown how parents may deal with the aforementioned trade-offs, and how these may influence the resolution of intense sexual conflict over care using Eurasian penduline tits as a model species. In *Chapters III and IV*, I argue that parents may benefit from concealing their intention to desert. The pattern of care in Eurasian penduline tits in itself implies that a decision of parental care is not independent of the partner, since biparental care does not occur (McNamara et al. 2002). If it were fully independent, biparental care would emerge by chance (in the range of about 2-14% of nests). Szentirmai et al. (2007) have pointed out that it is in the best interest of both parents to desert: both parents benefit in terms of reproductive success when they desert, rather than care for the offspring (see also *Chapter V*). This implies that both parents attempt to desert at the majority of nests. The likely result is that the timing of desertion becomes of utmost importance for

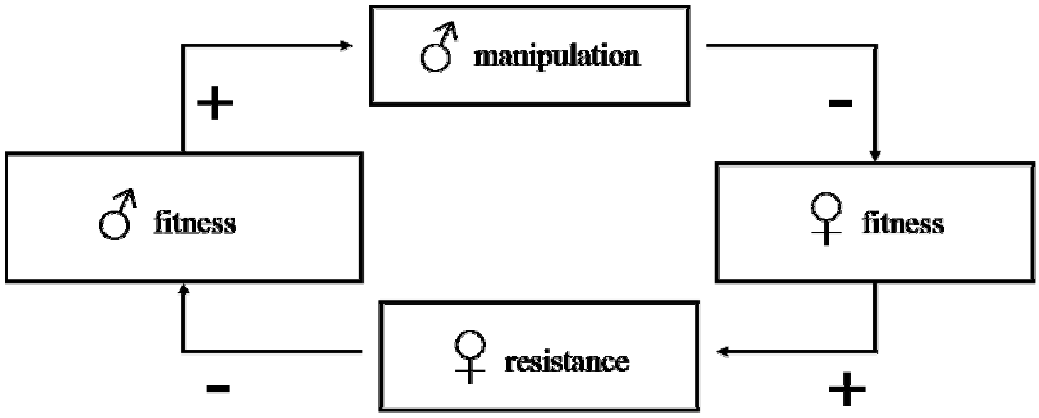


**Figure 10.1** The proposed antagonistic coevolutionary process of manipulation and resistance. Manipulation of the female by the male, e.g. exploiting the female's perception bias, likely reduces the fitness of the female. This will subsequently enhance the female's resistance against this manipulation, by being more selective among males, which will negatively affect the male's fitness. The latter will then select for more intense manipulative traits in males to overcome her resistance (based upon Dawkins & Krebs 1978; Holland & Rice 1998; Chapman et al. 2003).

both parents: deserting too early (or too late) will have important implications for both parents (see *Chapter III*), not the least the risk of being deserted by the partner (Lazarus 1990; McNamara et al. 2002). The latter will have a profound implication on the decision-making process of the deserted parent, since caring for the offspring results in reduced reproductive success (Szentirmai et al. 2007, *Chapter V*) and the alternative of biparental desertion causes the effort invested in the current brood to have been in vain. Therefore, bargaining over who deserts first is expected during a short time window when the female lays her eggs. In *Chapter III* and *IV*, using detailed behavioural observations, I showed that neither vocal nor nest building behaviour predicts which parent is going to desert the nest. Both studies also point out that the process of desertion is rapid, since (i) the timing of desertion is nearly always around the third day of egg laying, (ii) either parent may desert first, and (iii) at biparentally deserted nests both parents deserted within one or two days.

If desertion has the highest payoff for both sexes, this may explain the lack of cooperation between parents in the breeding system of Eurasian penduline tits. Such a situation where, in game-theoretical terms, defection appears to be the dominant strategy, is exemplified by the Prisoner's Dilemma. I tested the hypothesis that Eurasian penduline tits play the Prisoner's Dilemma in *Chapter V*. Using the seasonal reproductive payoffs for a given strategy, I concluded that desertion, although a beneficial strategy for both parents, is not the dominant parental care strategy. This makes intuitive sense, because this would result in biparental desertion

being the dominant parental care strategy in which case no offspring would survive. Hence, desertion is the most successful strategy, but only in case of unilateral desertion, i.e. one parent stays behind and cares for the offspring. This is a situation known among game theoreticians as the coordination game. These models are widely used by game-theoreticians and *Chapter V* is one of the few empirical explorations of these influential game models.

*Chapter V* also points out that a large proportion of the variation in parental care can be predicted from the strategy-dependent reproductive payoffs. Applying payoff dominance in a game-theoretic approach, i.e. those cases where there is no direct payoff dominance are resolved in a coordination game, I found proportions of male-only care, female-only care and biparental desertion that are similar to the observed proportions of care in our study populations. This is in congruence with my suggestion that there is little room for negotiations over parental in penduline tits: by and large the conflict is resolved directly via the expected payoffs. These results suggest that, although negotiations between the parents may play an important role in resolving conflicts (McNamara et al. 1999), this may only be true in those cases that are not directly resolved through the expected payoffs. If the expected payoffs for both players are countering each other, this may lead to failed negotiations. In the context of parental care, the latter may include biparental desertion in Eurasian penduline tits and little egrets, *Egretta garzetta* (Fujioka 1989).

Extra-pair paternity and egg-dumping can influence reproductive success, although in this thesis I did not include these. Provisional data suggest that, although extra-pair copulations do occur in Eurasian penduline tits such that about 36.5% of nests contain at least one extra-pair young, parentage does not seem to be associated with parental care decisions (Mészáros, LA, van Dijk, RE, van der Velde, M, Komdeur, J, Székely, T & Szabad, J. unpubl. data). Conflicting results exist from other passerine birds as to how parentage and parental care are related to each other. For example, male reed buntings, *Emberiza schoeniclus*, reduced their contribution to parental care with the number of extra-pair offspring in their nests (Dixon et al. 1994). However, a study that replicated the latter work in a different population of the same species found no evidence of reduced paternal care (Bouwman et al. 2005).

The complex breeding system of Eurasian penduline tits bears striking similarities to the dunnock, *Prunella modularis*. Both species exhibit variable mating and breeding systems within a population and this appears to be a result driven by the antagonistic interests of males and females (Davies & Houston 1986): Both in

penduline tits and in dunnocks, mating up with multiple partners enhances the reproductive success of the multiply mating parents, but decreases the amount of parental care at a given nest at a cost to the reproductive success of the partner (see Fig. 2.1, Fig. 10.2).

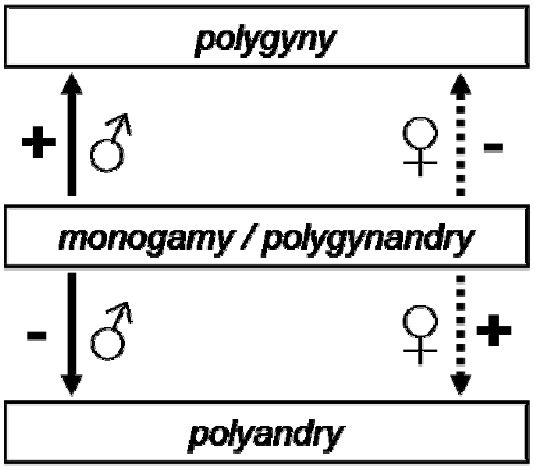


**Figure 10.2** Sexual conflict over mating in the dunnock. Both males and females benefit in terms of reproductive success from obtaining multiple mates, yet the reproductive success of each sex decreases with the level of polygamy of the other. Reproductive output is, on average, about equal for males and females in case of monogamy or polygynandry, although in polygynandry the reproductive success for males depends on whether the male is the dominant (α) or the subordinate (β). The optimal mating system of males is a mirror image of that of females (after Davies & Houston 1986).

There are, however, also some important differences between the two species: dunnocks, rather than attempting to desert the offspring and shifting all parental care to the mate, benefit from cooperating with multiple partners. Both sexes attempt to recruit several mates: the females do this to gain help in raising her offspring, whereas males gain if multiple females and foreign males feed his offspring. Thus, the conflict in dunnocks appears to be over mating, whereas in penduline tits the conflict is over care. Also, in the dunnock paternity appears to have an important influence on the amount of care a male should invest, such that paternity appears to be associated with the breeding system (Davies 1992), whereas in penduline tits cuckoldry does not seem to be related with the parental care strategy (Mészáros, LA, van Dijk, RE, van der Velde, M, Komdeur, J, Székely, T & Szabad, J. unpubl. data). In the dunnock various mating systems (polyandry, monogamy and polygynandry) may occur simultaneously (Davies 1992), whereas in penduline tits polyandry and polygyny occur largely sequential. A final, important difference between the two species can be found in the association of food availability with the breeding system. Davies and Lundberg (1984) showed that the ability of males to control access to females depended on size of the female's range. The latter was influenced by food availability, so that when food patches were dense, both naturally and

experimentally, female ranges were small and thus easily monopolised by males. This, in turn, opened up the opportunity for males to gain access to multiple females and thus polygyny and polygynandry could emerge. If food resources were limited, female ranges increased and so did the level polyandry. So it appears that the hard work parents have to deliver to find sufficient food for the offspring may drive the occurrence of the diverse breeding system. In penduline tits, however, I argue that the abundant food resources in conjunction with the good insulative capacities of the nest (Szentirmai et al. 2005b) has facilitated uniparental care, so that one of the parents may abscond from parental care and find a new partner (*Chapter VII*).

Different parental abilities of males and females may also influence care patterns. A sex difference in parental quality can be expected to exist for several reasons. Firstly, reproduction is physiologically a different process for the two sexes. The egg-laying window of females may be an important constraint on the timing of female nest desertion (see *Chapter IV*) and may have implications for the female's body reserves (see Bleeker et al. 2005). Secondly, males run the risk of being cuckolded and may thus benefit from mating with multiple females instead of allocating resources into providing parental care for a given brood (Trivers 1972; Davies & Houston 1986; Queller 1997; Kokko & Jennions 2008). Thirdly, the variance in reproductive success is often higher for males than for females (Queller 1997; Kokko & Jennions 2003). Females are thus expected to have evolved towards enhanced efficacy of parental care provisioning (Erckmann 1983; Eckert & Weatherhead 1987) and should thus be selected to provide parental care. Males, on the other hand, generally benefit more from remating than females, i.e. the Bateman gradient is steeper for males than for females (Bateman 1948; Andersson & Iwasa 1996) and thus males are selected to compete for mates.

In all populations of penduline tits in Europe studied to date, females consistently care more often (45-70% of nests) than males (5-20% of nests), and females care for, on average, larger clutches (Persson & Öhrström 1989; *Chapters VI and IX; Appendix I*). *Chapter VI* explored whether the preponderance of female-only care may be due to females being more efficient parents than males. However, the results did not support this expectation, because parental care provided by male and female penduline tits was not different, nor was offspring survival. Although the latter results need experimental testing, I conclude that the parental quality hypothesis cannot explain how the sexual conflict over parental care in Eurasian penduline tits is resolved. The latter result is in congruence with the suggestion that

parental care may largely consist of payoff based strategies: the sex difference in the reproductive payoffs likely is a major driver behind the sex difference in proportion of parental care strategies (Szentirmai et al. 2007; *Chapter V*).

# 2. Environment and parental care

*How does the environment influence a decision about parental care?* One of the central ideas in behavioural ecology is that resources influence mating systems and parental care (Verner & Wilson 1966; Emlen & Oring 1977; Davies 1991). An obvious resource is food. If food resources are plentiful, the offspring are more likely to survive. Rich resources, however, may also allow a single parent to raise the offspring unassisted (Bart & Tornes 1989; Székely et al. 1996; Olson et al. 2008; Eldegard & Sonerud 2009), opening up the opportunity for one of the parents to desert and enhance its reproductive output (Beissinger & Snyder 1987; Pilastro et al. 2001; Szentirmai et al. 2007). Given the fitness benefits, food availability is one of the key determinants of habitat quality.

However, both food availability and habitat quality are hard to assess accurately in the field. As a proxy I thus used the vegetation structure of the habitat to account for habitat quality variability that may influence parental care decisions (*Chapter VII*). Vegetation structure may also be associated with the availability of nest material, which may also influence the decision about parental care as nest size and nest building behaviour have been shown to play a role in this (Hoi et al. 1994; Szentirmai et al. 2005a, but see *Chapter III and IV*). I found that habitat structure was not associated with the parental care in Eurasian penduline tits, and suspect that the overall high food abundance may explain this: around each nest plenty of food and nest material is available. On an evolutionary timescale, however, abundant food resources may have been one of the drivers of the Eurasian penduline tit's variable breeding system. The latter proposition is supported by recent data on the breeding systems of other penduline tit species (Remizinae) and their habitats (see *Chapter IX and Appendix IV*, van Dijk, RE, Pogány, Á & Székely, T, unpubl. data). Previous studies in other species have found support for the proposition that food abundance may promote less cooperation between the sexes and thus increase levels of polygamy (Davies & Lundberg 1984; Leisler et al. 2002; Eldegard & Sonerud 2009).

A recent example illustrating such a relation between food availability and breeding system, is from the Tengmalm's owl, *Aegolius funereus*: when food

resources were abundant (both naturally and after experimental food supplementation) more, female uniparental, desertion took place and the females deserted the offspring earlier (Eldegard & Sonerud 2009). However, although many studies find some effect of food availability on breeding system, the generality of this association and the exact mechanism behind it are not clear and requires further research (e.g. Kosztolányi et al. 2006; Lin et al. 2006; *Chapters VII and VIII*).

Mating opportunity may also have a profound influence on the resolution of sexual conflict over care and the subsequent reproductive output. In *Chapter VIII* I investigated this by comparing the breeding system of Eurasian penduline tits across Europe, including a high-density population in Hungary and a sparser population in The Netherlands. Population density can be expected to influence mating systems in various ways, although the availability of mates is possibly the most profound one. Moreover, the strategy of deserting with a very low chance of remating and reproducing again seems to diminish the proposed fitness benefits of desertion drastically (Szentirmai et al. 2007; *Chapter V*). I concluded that, contrary to the expectation, the breeding system of Eurasian penduline tits is remarkably stable across different populations with different breeding densities throughout Europe. Given the magnitude differences in breeding densities between the sites, this has important implications on our understanding of how inflexible breeding systems may be between populations. On an evolutionary timescale the high breeding densities as observed in Central Europe, possibly driven by the abundant food resources, may have enhanced the evolution of the variable breeding system as high mating opportunities convey a fitness benefit (Szentirmai et al. 2007; *Chapter V*). However, once the population started to decline, or individuals have dispersed to low density areas, the breeding system may suffer from a positive feedback loop setting constraints on the success of the species (*Chapter VIII*). Understanding how the environment may drive the success of a species with a given breeding system may thus be important from a conservation point of view as well.

# 3. The evolutionary ramifications of sexual conflict

*How does sexual conflict over parental care influence the evolution of behaviour and morphology?* Sexual conflict is expected to be a powerful evolutionary force that may shape the evolution of behaviour and morphology and may promote speciation (Arnqvist et al. 2000; Martin & Hosken 2003, but see: Bacigalupe et al. 2007;

Wilkinson & Birge 2009). Evidence for these predictions, however, mainly stems from prezygotic sexual conflict, i.e. the different interests of males and females over mating (Martin & Hosken 2003). *Chapters III, IV, V* (see above) and in particular *Chapter IX* provided examples of how sexual conflict over parental care provisioning may impact upon behaviour and morphology of penduline tits. I have shown that Eurasian penduline tit parents appear to avoid each other at the nest so as to force their partner to attend the nest more often (*Chapter IX*). Additionally, sexual conflict over care is expected to be associated with intense sexual selection (Björklund 1990; Andersson 1994; Székely et al. 2007) due to a higher variance in reproductive success than in a cooperatively breeding species such as the Cape penduline tit. In accordance with this conjecture, I showed that Eurasian penduline tits exhibit more sexual dimorphism in plumage, and have a more complex song than Cape penduline tits. All a priori predictions derived from sexual conflict theory that I tested (see e.g. Chapman et al. 2003 and Houston et al. 2005) were supported in this study. I thus conclude that sexual conflict over care may have important ramifications on the evolution of morphology and behaviour. This is a novel and important addition to similar results stemming from sexual conflict over mating published in earlier studies (e.g. Arnqvist & Rowe 2002b; Anthes et al. 2008; Pizzari & Bonduriansky 2009).

Although we currently do not have evidence for a process of manipulation of the female's perceptive bias through exaggerated male ornaments (see Fig. 10.1), the fact that more attractive males in Eurasian penduline tits desert more often is consistent with this idea: females mated to attractive males pay a substantial cost by being deserted by their mate. Females are thus expected to evolve to be more selective among males, driving the evolution of male ornamentation. Another possibility is that females may retaliate against exploitation through this male manipulation by not caring more often than average for the offspring sired by more attractive males (*Chapter IX*). The rapid sequential desertion I found at biparentally deserted nests is consistent with this notion (*Chapter III & IV*), since quick desertion might be a counter strategy to avoid being exploited.

Why would females select attractive males if their reproductive success would thereby be suppressed? Also, why would attractive males desert if they anticipate that their partner will unlikely care? One possible answer to this conundrum may be that, although the seasonal reproductive success of females may be suppressed when mated to attractive males (Chapman et al. 2003; Kingma et al. 2008; *Chapter IX*), the female may gain indirect benefits through more manipulative

male offspring. In the latter scenario one would expect that, if females bias the sex ratio of the offspring, a male-biased sex ratio would occur in the offspring sired by attractive males, although this does not appear to be the case (*Appendix I*; see also: Cameron et al. 2003; Arnqvist & Kirkpatrick 2005; Chapman 2006).

# 4. Future directions

*The process of clutch desertion*

I suggest three major directions to further understand the process of desertion. Firstly, we need experimental manipulations. Experiments may be aimed at the different traits I have investigated in my thesis: random removal of the male or the female will provide insight into how the decision about parental care depends on the presence of the partner, whilst controlling for the effect of individual mate quality. I predict that removal of a random parent during egg-laying will increase the tendency for the experimentally deserted partner to stay behind and take up parental care (see *Chapters III and IV*). Additionally, such an experiment will provide insight into how the future benefits may depend on individual traits. Mating times, for instance, are expected to be longer for the randomly, experimentally removed parent than for those that deserted naturally if attractiveness of the parent influences the benefits of desertion, as I suggested in *Chapter IX*.

Experimental manipulation of clutch size will allow us to test if males and females are on average equally able to raise the offspring of large clutches. *Chapter VI* tested this proposition in a correlative manner, although the males that decide to care for the offspring may provide better care than an average male in the population. Experimental manipulation of clutch size and following the care patterns of manipulated versus control clutches would pre-empt this shortcoming.

Secondly, further theoretical work is necessary to understand how different variables may underlie mating and parental decisions building upon *Chapter V*. Using a state-dependent game theoretic model, parameterised with data collected from the field, such as on body condition (Bleeker et al. 2005) or male attractiveness (Pogány & Székely 2007; Kingma et al. 2008; *Chapter IX*), it will be possible to achieve a more accurate estimate of the proportions of payoff-based reproductive strategies. Theoretical models are also needed to analyse the timing of desertion: why do both males and females desert the nest when about three eggs have been laid? Incorporating the benefits and risks of deserting at different times may model the

theoretically optimal timing of desertion. An important risk factor for males will be the loss of paternity if he deserts too early (but see: Magrath et al. 2009). Such risks can also be parameterised using data collected from the field.

Thirdly, a better understanding of the mechanisms that drive the process of clutch desertion will provide important insights into physiological and genetic bases of parental care. I propose carrying out hormonal assays in males and females during the egg-laying period, i.e. the period during which desertion takes place. This should reveal which hormones are involved in the likely complex neuro-ethological pathways that influence care behaviour (see Adkins-Regan 2005). Experimental manipulation of hormones, on the other hand, has important benefits: it not only provides insight into which hormones may play a role in the process of clutch desertion, it will also allow us to investigate the interaction between the parents. How does a change in behaviour of one parent influence the behaviour of the other? Subcutaneous hormone implants, for instance blocking testosterone receptors or dispensing vasoactive intestinal peptide (VIP) to enhance a male's inclination to care for the offspring rather than to desert, have been used in other species in a different context and would seem a promising avenue to follow (Badyaev & Duckworth 2005).

Finally, we need to further our knowledge of the genetic basis of parental behaviour. Monitoring a year-round resident population would help to investigate the heritability of parental care and, possibly with the use of cross-fostering experiments, it would allow us to address whether offspring raised in male-only care nests is, for instance, more likely to provide male-only care in the subsequent breeding season, whereas offspring raised by the female-only may be more likely to adopt female-only care. These experiments can tease apart the influences of the social and non-social environment versus the genetic bases of parental care, although a full understanding will only emerge when nature and nurture are merged. Additional benefits of a resident population would be the ability to investigate age effects on parental care, and to what extent indirect benefits for males and females may drive sexual selection in penduline tits. Both have so far been impossible given the low adult returning rates between years (see *Chapter VII*).

*Environment and parental care*

I envisage experimental manipulations of environmental resources are necessary to test how these may influence the resolution of sexual conflict about parental care. In

the field this will not be straightforward, although captive populations may offer opportunities for experimental supplementation of food and/or nest material, and to simulate the absence or availability of potential future partners investigating how this influences how males and females decide to care for or to desert the offspring.

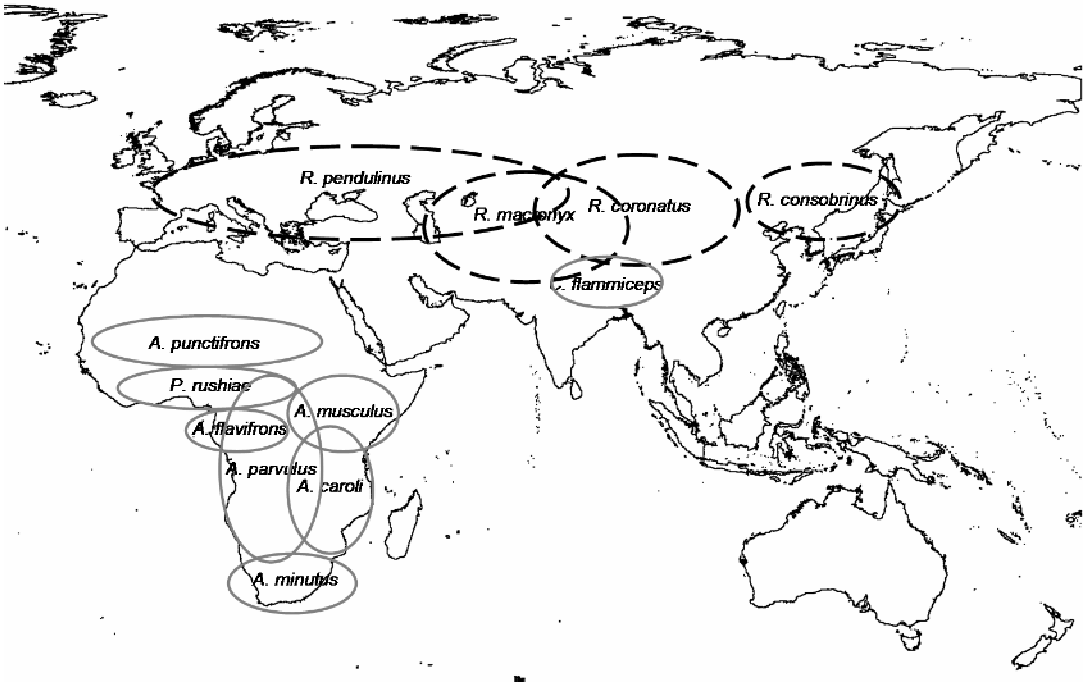


**Figure 10.3** Distribution of penduline tits (Remizina*e*, 13 species, 5 genera, Madge 2008). The verdin, *Auriparus flaviceps*, often included within the Remizinae subfamily Madge 2008, is not displayed on this map. It occurs in the South of the USA and in the North of Mexico. However, recent molecular sequences suggest that the verdin, the tit-hylia (*Pholidornis rushiae*) and the fire-capped tit (*Cephalopyrus flammiceps*) should not be included within the Remizinae (van Dijk, RE, Irestedt, M, Ericson, P and Székely, T, unpublished data). The grey, continuous ellipses of distribution indicate the species that exhibit biparental care only. The species surrounded by a dashed, black ellipse may exhibit uni- or biparental desertion. Note that the breeding biology of the majority of the species is poorly described (Harrap & Quinn 1996).

*The evolutionary ramifications of sexual conflict*

We need comparative studies to further our knowledge of the ramifications of sexual conflict over breeding systems at various levels. Detailed studies of closely-related species will be invaluable. Penduline tits are excellent study systems for these purposes: they exhibit a full range of breeding systems from intense sexual conflict over parental care with polygamy by both sexes in Eurasian penduline tits, through uniparental, female-only care in Chinese penduline tits, *Remiz consobrinus* (van Dijk, RE, Pogány, Á, & Székely, T, unpubl. data), to facultative cooperative breeding in Cape penduline tits (*Chapter IX; Appendix II, IV*). Additionally,

penduline tits occur in different habitats, ranging from reed marshes with abundant food through to dry, coastal scrubland in South Africa and oases in the Gobi desert in China (Harrap & Quinn 1996; *Chapter IX*; *Appendix IV*; van Dijk, RE, Pogány, Á & Székely, T, unpubl. data; Fig. 10.3). This subfamily will allow us to investigate (i) how different intensities of sexual conflict are associated with behaviour and morphology and (ii) how different breeding systems may have evolved as driven by various environmental characteristics. Including several species as discussed in *Chapter IX* will also allow us to test my results based upon two species of penduline tits, and to control statistically for potentially confounding variables.

A challenging project is to superimpose the impact of sexual conflict at the behavioural and morphological level onto a phylogenetic tree (van Dijk, RE, Irestedt, M, Ericson, P & Székely, T, unpubl. data) will allow us to investigate the rate of evolution of various traits, rates of diversification within a confined group of species, and determine which traits are likely to drive speciation. The latter may subsequently be experimentally tested in the field.

Taken together, the tame behaviour, the accessible breeding ecology and the amenability of penduline tits offer lots of opportunities for experimental studies. I also feel that intense study of a resident penduline tit population can address some of the key evolutionary issues, such as intensity of selection and heritability of traits associated with breeding systems. Finally, phylogeographic analyses of Remizinae will likely advance not only understanding of breeding systems, but also the actions of sexual conflict and parental cooperation in the wild.

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# APPENDIX

# I

## OFFSPRING SEX RATIO IN THE SEQUENTIALLY POLYGAMOUS PENDULINE TIT *REMIZ PENDULINUS*

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*Journal of Ornithology (2008) 149: 521-527*

*Authors' contributions*

**REvD**: study design, data collection, statistical analyses, manuscript preparation

**JK**: manuscript improvement

**MvdV**: molecular sexing, manuscript improvement

**IS**: data collection, manuscript improvement

**XY**: molecular sexing

**RfC**: molecular sexing, manuscript improvement

**TS**: study design, manuscript improvement

**ABSTRACT**

Despite the growing literature on facultative sex-ratio adjustment in chromosomal sex-determining vertebrate taxa (birds, mammals), the consistency of results is often low between studies and species. Here, we investigate the primary and secondary offspring sex ratio of a small passerine bird, the Eurasian penduline tit (*Remiz pendulinus*) in three consecutive years. This species has a uniquely diverse breeding system, in which the male (and/or the female) abandons the nest during egg-laying, and starts a new breeding attempt. This allowed us to test (i) whether patterns of parental care, i.e. male-only care, female-only care or biparental desertion, influence offspring sex ratio, and (ii) whether the offspring sex ratio is repeatable between successive clutches of males and females. Using molecular markers to sex 497 offspring in 176 broods, we show that (i) offspring sex ratio does not depend on which parent provides care, and (ii) the offspring sex ratio is not repeatable between clutches of a given individual. The overall primary and secondary offspring sex ratio at a population level is not different from parity (54 ± 6% males, and 50 ± 3% (mean ± SE), respectively). We suggest that ecological and phenotypic factors, rather than individual traits of parents, may influence offspring's sex, and conclude that there is currently no evidence for a facultative adjustment of offspring sex ratio in the penduline tit.

## INTRODUCTION

Fisher's (1930) frequency-dependent model of sex allocation predicts that natural selection will maintain an even population sex ratio as long as the cost of producing a male is equal to that of producing a female. However, the optimal offspring sex ratio of an individual parent may deviate from parity due to a variety of factors resulting in a relative fitness difference between sons and daughters (Trivers and Willard 1973; Hasselquist and Kempenaers 2002; Komdeur and Pen 2002; West et al. 2002). The recent advent of molecular sexing techniques has contributed significantly to the question whether mothers should bias the sex ratio of their offspring. In contrast to previous work (Charnov 1982; Clutton-Brock 1986), recent studies suggest that animals with chromosomal sex determination, such as birds and mammals, are indeed able to facultatively adjust the primary sex ratio of their offspring in an adaptive manner (Komdeur and Pen 2002; Hasselquist and Kempenaers 2002; West et al. 2002; West and Sheldon 2002). For example, females may bias the sex ratio of their offspring in response to the attractiveness of the father (Kempenaers et al. 1997; Sheldon et al. 1999), the timing of breeding (Daan et al. 1996; Badyaev et al. 2003; Székely et al. 2004), levels of parental care (Clutton-Brock 1991), and to biased population sex ratios leading to more intense competition for mates among one sex (Hamilton 1967). However, despite several studies reporting biases in offspring sex ratio, others failed to find deviations from parity (e.g. Saino et al. 1999; Grindstaff et al. 2001; reviewed in Komdeur and Pen 2002). This underlines the need for further studies including those reporting non-significant effects as well as significant findings to avoid publication bias (Festa-Bianchet 1996; Cockburn et al. 2002; Hasselquist and Kempenaers 2002).

Griffin et al. (2005) argue that across species the selection on sex-ratio adjustment may be variable due to differences in breeding system, sexual dimorphism, and life-histories, and as such cause biological variation in sex-ratio adjustment, whereas within species such variability in selection is less straightforward. Studies investigating the repeatability of facultative sex ratio adjustment in the same species between years, and for the same individuals are scarce, yet crucial to determine with confidence the frequency of sex-ratio modification in specific taxa (Palmer 2000; Ewen et al. 2004; Cassey et al. 2006; Korsten et al. 2006).

Here we investigate sex allocation in Eurasian penduline tit (*Remiz pendulinus*) in three consecutive breeding seasons. The penduline tit is a small

passerine (body mass about 9g) with modest sexual dimorphism: adult males have brighter plumage and larger eye-stripes than females (Cramp et al. 1993; Glutz von Blotzheim and Bauer 1993; Kingma et al. 2008). Penduline tits have highly diverse breeding strategies that involve sequential polygamy by both sexes, and uniparental incubation and subsequent brood care by either the male (5-20% of nests) or the female (50-70% of nests). An unusual feature of penduline tit breeding biology is the high frequency of nest desertion by both parents (approximately 30% of clutches) dooming these eggs to failure (Franz 1988; Persson and Öhrström 1989; Szentirmai et al. 2007). Since desertion takes place during egg laying, clutches cared for by the male and clutches deserted by both parents are usually smaller (3.4 ± 1.3 eggs (mean ± SD)) than clutches cared for by the female (5.9 ± 1.3 eggs), since the female may lay a few more eggs after her mate has deserted (Franz 1991; Persson and Öhrström 1989).

The breeding system of penduline tits allowed us to address three major objectives. Firstly, we tested whether offspring sex ratio depends on which parent provides care, by comparing offspring sex ratios of male-only cared clutches with those in female-only cared and biparentally deserted clutches. The sex of the offspring may vary with laying order in several birds (*e.g.* Kilner 1998, Komdeur et al. 2002, Cichoń et al. 2003), and given that desertion takes place during egg-laying in penduline tits, this may lead to a different sex ratio in male-only cared and female-only cared clutches. Since the proportion of sons has been reported to increase with laying order (Kilner 1998, Krebs et al. 2002), we expected female-only cared clutches to be more male-biased than male-only cared clutches in penduline tits, since females usually lay 2-3 more eggs after the male deserted.

Secondly, we expected repeatable sex allocation between successive nests of a given individual if individual characteristics, such as attractiveness or parental abilities, influence the offspring's sex, and is independent of the quality of the mate, territory and season (but see Oddie and Reim 2002). To investigate how variation in brood sex ratio may depend on individual characteristics, we calculated the repeatability of offspring sex ratio of individual males and females that produced several broods in a given season.

Thirdly, we tested whether offspring sex ratio deviated from unity at the population level and whether the distribution of sons was different from binomial distribution. The rationale behind the latter was that even if offspring sex ratio is not different from unity at population level, some females may produce largely sons

whereas others largely daughters, and deviation of these extreme phenotypes may be different from the binomial expectation (e.g. Radford and Blakey 2000, Westneat et al. 2002, Dietrich-Bischoff et al. 2006).

## METHODS

*Fieldwork*

We studied the penduline tits at an extensive system of fish-ponds near Szeged in southern Hungary (46°19'N, 20°5'E) where they breed along the dikes which separate the ponds. Fieldwork was carried out between April and August 2002 – 2004. A total of 214, 183, and 178 nests was found in 2002, 2003, and 2004, respectively. Males start building their nest and sing to attract a female, although it takes 8.9 ± 7.0 days for a male to find a mate ($n$ = 111 males). Of all nests 52% was abandoned either before pair formation had taken place (i.e. the male was unsuccessful in attracting a female (37%)), or the nest was abandoned due to disturbance by humans, heavy winds or predation (12%), or a new owner overtook the nest (4%)). We searched the study site to identify unpaired, nest-building males, and then visited the males every other day to monitor their status by observing them for at least 15 minutes (see details in Bleeker et al. 2005, Van Dijk et al. 2007).

We collected the following data at each nest: (i) Nest initiation date. The exact date of initiation could be determined for nests found when only a small amount of nest material is woven around a twig (categorized as stage A, see fig. B on p. 385 in Cramp et al. 1993). Initiation dates for nests found in later stages (stages B-E; see fig. C-I on pp. 386-387 in Cramp et al. 1993) were estimated by comparison with the progress of nests that had been followed continuously since stage A (Szentirmai et al. 2005). (ii) Date of pair formation. A male was considered paired if he was seen copulating with a female near the nest, or the pair was observed building the nest together. (iii) Sex of attendant parent. We identified which parent was attending the nest at each stage of the nesting cycle (nest building, egg-laying, incubation, nestling period). A parent was considered to have deserted the nest if it had not been seen during at least two consecutive nest checks. Birds classified as 'deserted' were never seen at the nest subsequently. (iv) Start of incubation. This was determined by observing the behaviour of the parent: incubating parents stay inside the nest for longer continuous periods then nest building birds.

Adults were individually marked using a unique combination of three colour-rings, and one numbered metal ring from the Hungarian Ornithological Institute.

Returning rates of adults are low across years: out of 195 colour-ringed males from 2002 and 2003, only 13 males were resighted in one or both subsequent years. Similarly for females: out of 87 colour-ringed females, only 8 were resighted. A small blood sample (about 10μl) was taken from adults and 10-day-old nestlings by puncturing their brachial vein. Unhatched eggs in all clutches including the incubated ones were checked for the presence of an embryo. Clutches deserted by both parents were taken to the laboratory and incubated indoors using an incubator set at 37.5°C. Eggs were opened after being incubated for five days and any visible embryos were placed in an Eppendorf tube.

*Molecular sexing*

For nestlings DNA extraction was carried out using the GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences Corp. USA). DNA from egg-samples was extracted using the Chelex method (Walsh et al. 1991). The sex of the offspring was determined by DNA amplification using P2 and P8 primers for PCR under the reaction conditions given in Griffiths et al. (1998). We blindly repeated the molecular sexing of 26 nestling and 14 egg samples chosen at random: all matched the sex assigned by the first test. Using DNA collected in 2005 we also compared the molecular sexing of 22 adults (14 males, 8 females) with the sexing done in the field based on plumage and behavioural characteristics. All adults were sexed consistently with our field observations.

*Statistical analyses*

We investigated repeatability of offspring sex ratios for males and females using bootstrapping, since parametric estimation (Lessells and Boag 1987; Harper 1994) was not feasible given that the proportion of sons was not normally distributed and the variances were heteroscedastic. We calculated the within-year repeatability of offspring sex ratio by choosing males (or females) that had multiple nests in a given season. We only include within-year repeatabilities and not between years, because returning rates are low (see above). First, we calculated the absolute mean difference in the proportion of sons between all nests of a given parent, and took the mean of these individual means (δ test statistic). Second, the sex of the offspring was randomised $10^4$ times by keeping the original data structure. At each iteration we calculated δ as for real data. Third, we calculated the proportion of cases in which the randomised values were less than the test δ. We report the test statistic and the

probability of finding a value smaller than or equal to the test δ. Randomization was carried out by Resampling Stats™ for Excel version 3.2 (2006).

All analyses were carried out using three sets of data. First, we used the full dataset that included all nestlings and unhatched eggs. Second, we repeated the analyses separately after dividing the dataset into eggs and nestlings. We provide the results of both given the interest in primary (eggs) and secondary (nestlings) sex ratios. Third, we investigated the influence of which parent, male, female or none, provided parental care on offspring sex ratio. If several nests were available for an individually marked male or female, we selected one nest randomly to avoid pseudoreplication, under the condition that male-only cared nests were selected in priority to female-only or biparentally deserted nests due to the limited number of male-only cared nests (12.4% of nests). At 42 of randomly selected nests of individually marked males the female was unringed. At 18 of these nests multiple females bred at the same time so these clutches were produced by different females. For the remaining 24 nests where the female was unringed pseudoreplication cannot be excluded, although we suspect it is small given (i) the size of our breeding population (see above), (ii) the fact that offspring sex ratio is not repeatable between nests of given individuals (see Results), and (iii) mate fidelity is low and remating between adult breeders is extremely rare.

We used generalised linear mixed models (GLMMs) with binomial error distribution and a logit link function to test the influence of parental sex on offspring sex ratio of individual eggs and nestlings nested within a brood using R (2005). The GLMM used the sex of each egg or nestling as the unit of analysis, the type of parental care (male-only, female-only or biparental desertion) as the explanatory variable, and brood ID as a random factor. The dispersion parameter was set to 1.0. Date of pair formation and year were also included in the model to test for an influence of season or year on the effect of parental care type. To assess the influence of parental care we used the Wald statistic, which has an approximately chi-square distribution. We considered using parent ID as a random factor in the analyses, but rejected this idea because for many broods the parents were not ringed (in 47% of 176 broods the male ID, female ID or both were unknown). More females than males were unringed, and unringed females were especially common in male-only cared and biparentally deserted nests, since females were usually trapped during incubation.

At the population level, we tested whether the proportion of sons deviates from 0.5 using a one-sample Wilcoxon signed-ranks test in MINITAB® release 12.2 (1998). To test whether the offspring sex ratio deviates from the binomial distribution (Sokal and Rohlf 1995), we calculated the number of males for all nests with equal number of sexed eggs and/or chicks and compared the observed frequencies with the expected ones using a $\chi^2$-test. Nests with between two and six sexed eggs and/or chicks were included in the latter analysis

Data are represented as means ± SE, and we provide two-tailed probabilities. Statistical significance was judged at the 0.05 level.

## RESULTS

The attendant father was identified at 95 out of a total of 176 nests sampled for offspring sex determination. The attendant mother was known at 85 out of the 176 nests. Of those known males 41 produced several broods within a year (mean: 2.41, range: 2-5 broods), compared with 19 individually marked females (mean: 2.21, range: 2-4 broods). We sampled 64 nests in 2002 (a total of 24 eggs and 152 nestlings), 55 nests in 2003 (37 eggs and 104 nestlings), and 57 nests in 2004 (36 eggs and 144 nestlings); in total 497 offspring (97 eggs and 400 nestlings) in 176 nests. These included both partial and complete clutches and broods (Fiala 1980). For 57 nests only eggs were analyzed, of which 8 complete and 49 partial clutches. For 115 nests only nestlings were analyzed, of which 88 complete and 27 partial broods. For 4 nests both eggs and nestlings were analyzed. Of each of these nests a sub-sample of the full clutch or brood was included in the analyses.

### *Offspring sex ratio and parental care*

Out of 169 nests 21 (12.4%) were cared for by the male, 103 (60.9%) by the female, and 45 (26.6%) nests were deserted by both parents. At seven nests the sex of the attendant parent was not known. Offspring sex ratio did not differ between male-only care, female-only care and biparentally deserted nests, when both eggs and nestlings were included in the analyses, (51±7%, 42±4%, 50±10%, respectively; $\chi^2 = 2.04$, d.f. = 2, $P = 0.36$, $n = 247$ offspring in 90 broods). This result remained consistent when the analysis was restricted to nestlings, thereby, per definition, excluding biparentally deserted nests: 56±7%, 43±4%, respectively; $\chi^2 = 1.11$, d.f. = 1, $P = 0.29$, $n = 202$ offspring in 62 broods. We did not find an effect of year or date of pair formation when these were incorporated into the model ($P > 0.31$).

*Repeatability*

The proportion of sons was not different from random between nests of a given male (51±4%; δ = 0.35, $P = 0.14$, $n = 37$ males), or of a given female (40±6%; δ = 0.45, $P = 0.76$, $n = 15$ females; nests include both eggs and nestlings). Given that there was no effect of parental care on offspring sex ratio (see above), we did not control for a potential effect of parental care on repeatability. Sample sizes did not allow us to analyse eggs and nestlings separately.

*Offspring sex ratio*

252 out of 497 offspring (51%, pooling unhatched eggs and nestlings), were male. Of the 97 unhatched eggs 52% was male, compared to 51% of 400 nestlings; therefore, the overall sex ratios did not differ from parity (Table I.1a). The latter results stand when the analysis was restricted to the average proportion of sons of individually marked males (Table I.1b) or females (Table I.1c), suggesting that pseudoreplication unlikely influenced the results.

Offspring sex ratios were not different from binomial distribution (brood size = 2, $n = 38$ nests, $P = 0.623$; brood size = 3, $n = 33$, $P = 0.424$; brood size = 4, $n = 32$, $P = 0.849$; brood size = 5, $n = 15$, $P = 0.753$; brood size = 6, $n = 11$, $P = 0.794$).

## DISCUSSION

Offspring sex ratio in the penduline tit does not depend on which parent provides brood care, is not repeatable between broods of individual males or females, and does not deviate from parity considering the population as a whole. It also does not deviate from the binomial distribution, which suggests that there is no bias within broods towards male- or female-only offspring. Palmer (2000) and Ewen et al. (2004) criticise the reported deviations from a 1:1 primary sex ratio and the evidence that birds are able to modify their sex ratio in an adaptive manner (but see: Hasselquist and Kempenaers 2002). However, Cassey et al. (2006) argue that an overall trend in facultative adjustment of offspring sex ratio is weak but significant using the same data as Ewen et al. (2004). Several examples of adaptive offspring sex-ratio adjustment in birds have been reported (e.g. kestrel *Falco tinnunculus*, Dijkstra et al. 1990; Seychelles warbler *Acrocephalus sechellensis*, Komdeur et al. 1997; blue tit *Cyanistes caeruleus*, Sheldon et al. 1999, Korsten et al. 2006). Since these taxonomically diverse bird species appear to adjust offspring sex ratios, it

**Table I.1** Percentage of sons in Eurasian penduline tits in all samples (a), and the percentage of sons of individually marked males (b), and of individually marked females (c).

(a)

| | % of sons (mean ± SE) | $z^a$ | *P* | *n* (nests) |
|---|---|---|---|---|
| Eggs only | 54 ± 6 | 950.5 | 0.46 | 61 |
| Nestlings only | 50 ± 3 | 1979.5 | 0.93 | 119 |
| Eggs & Nestlings | 52 ± 3 | 5311.0 | 0.44 | 176 |

(b)

| | % of sons (mean ± SE) | $z^a$ | *P* | *n* (males) |
|---|---|---|---|---|
| Eggs only | 45 ± 8 | 156.0 | 0.63 | 29 |
| Nestlings only | 47 ± 3 | 365.5 | 0.40 | 62 |
| Eggs & Nestlings | 46 ± 4 | 985.5 | 0.34 | 91 |

(c)

| | % of sons (mean ± SE) | $z^a$ | *P* | *n* (females) |
|---|---|---|---|---|
| Eggs only | 38 ± 12 | 37.0 | 0.35 | 14 |
| Nestlings only | 53 ± 3 | 990.5 | 0.30 | 73 |
| Eggs & Nestlings | 51 ± 3 | 1240.5 | 0.68 | 85 |

[a] Test statistic of Wilcoxon one-sample tests using 50% as test-median. Sex ratio per nest is used as datum.

seems unlikely that penduline tits are genetically or physiologically unable to do so. However, Griffin et al. (2005) suggested that variation in the occurrence of sex-ratio adjustment might be explained by the strength of selection for such adjustment, which differs across species. In the penduline tit there may not be a clear fitness benefit of offspring sex-ratio adjustment. There is no clear sexual size dimorphism and thus the costs of raising male offspring is unlikely to be very different from that of raising female offspring. Furthermore, not only males but also females may obtain several mates within a given season (Szentirmai et al. 2007). So the reproductive success of male and female parent not only increases with the number of females their sons will mate with, but also with the rate of polygamy of their daughters. Similarly, if attractiveness would be heritable, this may involve a fitness advantage

for both male and female offspring. Selection for adjustment of offspring sex ratio may therefore be weak in this species.

Our study also shows that, using data from three years there is currently no evidence for a facultative adjustment of offspring sex ratio in penduline tits. This means that even if under certain conditions there may be a bias in offspring sex ratio, the frequency and robustness of such a bias in this species is likely to be low. This may have implications for the way we study sex allocation in vertebrates: in order to understand the evolution of adaptive sex-ratio modification, an unbiased literature with repeated studies, between years and within years between given individuals, is of primary importance. In this study we show that the offspring sex ratio in successive broods of a given individual male or female is not repeatable, and that the distribution of males and females within broods is not different from the binomial distribution. These notions suggest that overall individual quality or individual traits such as plumage colouration or condition, may not play an important role in the determination of an offspring's sex. This is in line with most previous studies investigating repeatability of sex ratio within years (e.g. Leech et al. 2001; Westneat et al. 2002; Dietrich-Bischoff et al. 2006), although also here contrasting results have been reported (see for instance Whittingham et al. 2005). The selection to produce more sons when mated to an attractive male, for instance, may be weak and counteracted by increased costs of producing attractive males or costs of manipulation itself (Fawcett et al. 2006). Rather, ecological factors such as temperature (Badyaev et al. 2003), territory quality and/or breeding opportunities may be paramount (Komdeur et al. 1997; Hipkiss and Hornfeldt 2004). For the latter, comparing different populations of penduline tit may provide new insight, as at different sites across Eurasia habitat quality and, in particular, breeding opportunities are likely to vary widely (D.M. Brinkhuizen, R.E. van Dijk, T. Székely, J. Komdeur. unpublished data). Recently, Griffin et al. (2005) showed that cooperative breeding species in general adaptively biased sex ratios towards the helping sex, depending on the benefits that can be gained from having helpers around. To build on the present study, it would be of particular interest to investigate sex ratios in Cape penduline tit *Anthoscopus minutus*. This species is assumed to be closely related to the Eurasian penduline tit, yet exhibits facultative cooperative breeding (Dean 2005) – in sharp contrast to the breeding system of the species investigated in the present study. This may shed further light on the link between the evolution of sex-ratio adjustment and cooperation (Griffin et al. 2005).

**ACKNOWLEDGEMENTS**

We would like to thank the people that assisted in the field work: Maarten Bleeker, Dušan Brinkhuizen, Kenneth Hayes, Péter Horváth, Arno wa Kang'eri, Sjouke Kingma, Otília Menyhárt and Lídia Mészáros. We also acknowledge Kiskunság National Park and Szegedfish Ltd. for permission to carry out our fieldwork at Fehértó. The manuscript benefited from comments on earlier drafts by Tim Fawcett, Thomas Friedl, and one anonymous reviewer. The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002-2006) under contract n. 28696. Further financial support was provided by a University Studentship of the University of Bath to REvD, and grants from the Hungarian National Science Foundation (OTKA T031706, T043390) and a Royal Society Joint Project grant (15056) to TS. TS was also supported by The Leverhulme Trust (RF/2/RFG/2005/0279) and a HRDY Visiting Fellowship of Harvard University. The fieldwork carried out for this study complies with the Hungarian law.

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# APPENDIX

# II

## STRATEGIC DIFFERENCES
## THE BATTLE OF THE SEXES IN PENDULINE TITS

**René E. van Dijk & Ákos Pogány**

*Africa – Birds & Birding (2008) June/July: 52-55*

# strategic differences

## THE BATTLE OF THE SEXES IN PENDULINE-TITS

TEXT BY RENÉ E. VAN DIJK & ÁKOS POGÁNY
PHOTOGRAPHS BY RENÉ E. VAN DIJK

Birds adopt a wide variety of approaches to raising young, and why breeding strategies differ among species remain some of the central questions in ornithology. In a large number of birds, both parents care jointly for their offspring. Some species even have more extended cooperative breeding strategies in which additional birds, referred to as 'helpers' or 'alloparents', assist the breeding pair. Limited availability of food, combined with a relatively harsh climate and high predation rates, are thought to be important factors which drive cooperation in parenthood.

The Cape Penduline-Tit *Anthoscopus minutus* is such an example: both parents of this southern African near-endemic incubate and feed their young, and may sometimes be assisted by helpers, usually offspring from a previous brood.

In the northern hemisphere, however, such cooperation is rarer. The Eurasian Penduline-Tit *Remiz pendulinus*, a close relative of the Cape Penduline-Tit, is anything but cooperative and, in fact, it provides one of the best examples of sexual conflict over parental care among birds. In this species, one of the parents (usually the male) deserts its partner during the egg-laying period, leaving it to incubate the eggs and raise the chicks alone. By abandoning its partner and the clutch, the deserting parent not only saves on the costs of parental care, it benefits by gaining the opportunity to find another partner with which it can start a new nest, thus enhancing its annual reproductive success. Both males and females may have up to six mates in a given breeding season.

The downside of this promiscuous breeding system, however, is that the sexual conflict results in approximately a third of all clutches being abandoned by both parents during the egg-laying phase. The effort of building the elaborate nest and laying eggs are thus nullified as the parents are not there to care for the eggs.

Such sexual conflict is based on the fact that parental care, although beneficial for the offspring, is costly in terms of time and energy for both parents. Each individual parent is therefore better off transferring these costs to the partner, since it will gain the

fitness benefit of passing on its genes to the next generation, but with minimal costs to itself. In the reed-marsh habitats of Europe, where the Eurasian Penduline-Tit lives, food is plentiful, so a single parent may be able to raise the offspring successfully. This food-rich environment, combined with a relatively mild climate and low predation rates, may have promoted the evolution of this breeding system.

Since 2002, our team of researchers from the universities of Bath (UK), Budapest (Hungary), and Groningen (The Netherlands), headed by Professor Tamás Székely, has been investigating the Eurasian Penduline- Tit's unique breeding system in Hungary. We found that both males and females produce more offspring in a year by deserting the brood and leaving their partner to care for the young, emphasizing the intensity of sexual conflict.

**Above** *The nest of a Cape Penduline-Tit has a false entrance (the pouch underneath the spout). The spout is usually closed, to prevent snakes from gaining access to the young*

.

**Right** *Koeberg Nature Reserve, a fynbos habitat which contains relatively little food for Cape Penduline-Tits.*

**Opposite** *The male and female Cape Penduline-Tit look similar. They have only a thin eye-stripe, and both sexes have a similar grey back.*

The males vary in the size of their black facial mask: the larger it is, the more attractive they are to females. The males that were most successful in deserting one partner and finding a new one turned out to be the more 'attractive' males, with larger masks. A female that mates with an attractive male is therefore more likely to be left behind to bear the brunt of care, illustrating some of the costs a female faces when mated to an attractive male. We also found that parents made snap decisions, with desert/remain choices occurring during the course of a single day.

Also, partners do not reveal their level of commitment to the other bird. It seems that parents disguise their intention to care for or desert their offspring, and try to abscond before their partner does so.

The latter results led us to think that a sexual conflict over parental care may be apparent in other, unrelated behaviour patterns. Additionally, the recently 'rediscovered' sexual- conflict theory predicts that such an intense conflict leaves evolutionary footprints in traits, such as plumage and song, that are selected for by females when choosing a partner. Could this have been the case in penduline-tits?

It seems that parents disguise their intention to care for or desert their offspring, and try to abscond before their partner does so

To find out, in a collaborative study with Dr Penn Lloyd of the Percy FitzPatrick Institute at the University of Cape Town, we went to Koeberg Nature Reserve in the Western Cape to examine the breeding system of the Cape Penduline-Tit.

The two penduline-tit species are strikingly similar in several respects, particularly in size (both weigh less than 10 grams), in having a sharply pointed conical bill, in the efficient use of their feet when gleaning foliage for food ▷

*A female* (top) *and male* (above) *Eurasian Penduline-Tit at their nest. The male has a much larger mask, a more deeply coloured red-brown back, and has red spots on his breast. The female is altogether much paler.*

and, most obviously, in their elaborate, domed nests, which are built with an entrance spout. However, the two penduline-tit species have a fundamentally different approach to parenthood. Such contrasting breeding systems between closely related species is unusual in the bird world, but it offered us a good opportunity to study whether this may have had its predicted evolutionary impact on the species' behaviour and plumage.

While we were doing fieldwork at Koeberg, we noticed that the climate seems harsher, with lower night temperatures than in Hungary, that the risk of nest predation is much higher, and that food in this fynbos habitat seemed much less abundant than in the reedmarshes of Europe. These differences may have constrained the opportunity for sexual conflict to emerge in Cape Penduline-Tits, because both parents may be required to keep the eggs at the right temperature during incubation, to guard against potential predators and to find sufficient food for the offspring.

We observed that Cape Penduline- Tit partners behaved differently to their European counterparts. Cape Penduline-Tit pairs typically arrive at and leave the nest together, and we also saw them allopreening, a cooperative behaviour we have not observed in Eurasian Penduline-Tits. During the egglaying phase, we carried out detailed behavioural observations with a digital handycam, which photographed the nest every five seconds during the day. This provided in-depth data on nest attendance by partners, which had been colour-ringed for individual identification.

We had used the same method in Hungary to monitor nest attendance by Eurasian Penduline-Tits during the crucial phase in which either or both parents desert the nest. What we discovered was that Cape Penduline-Tit parents not only spend more time together at the nest, but that they also spend more time attending the nest than Eurasian Penduline-Tits. This is in line with the expected effect of sexual conflict on the behaviour of penduline-tits. Greater sexual conflict in the Eurasian Penduline-Tit means that parents try to avoid each other at the nest in order to force their partner to work harder, whereas Cape Penduline-Tits are more cooperative on the basis of their shared commitment to parenting.

In addition, to further test predictions of the sexual- conflict theory, we recorded the songs of all males and took digital photographs of the heads and backs of all trapped Cape Penduline-Tit parents. This enabled us to compare the song and the plumage with that of Eurasian Penduline-Tits. Sexual-conflict theory predicts that these traits (song and plumage), which the females seek out when picking a partner, should be more elaborate in the species exhibiting more intense sexual conflict, and that those species should be more sexually dimorphic.

Consistent with that theory, we found that Eurasian Penduline-Tit males have far more intricate songs than Cape Penduline-Tits: they use some 16 different 'notes' in their song, whereas the

Cape Penduline-Tit males sing only a single 'note'. Eurasian Penduline-Tits also exhibited a clear sexual plumage dimorphism, with the male having a larger eyemask and a more colourful back than the female, whereas male and female Cape Penduline-Tits appear identical.

So, in essence, the differences that we found between the species are consistent with our predictions based on the difference in breeding system. The level of cooperation in breeding is mirrored in the level of cooperation in behaviours such as nest attendance.

But why do male and female Eurasian Penduline- Tits show such sexually dimorphic plumages, whereas Cape Penduline-Tits males and females are similar? This can be ascribed to a process known as 'sexually antagonistic coevolution', which only becomes readily apparent in species experiencing sexual conflict. Over evolutionary time scales, attempted manipulation by males is countered by resistance or retaliation by females. Males might thus exploit a female mate preference for a fancier appearance

As an evolutionary response, females retaliate, becoming choosier about male traits in order to pick the mate of highest quality

or song. As an evolutionary response, females retaliate, becoming choosier about male traits in order to pick the mate of highest quality: is the male that looks good and sings beautifully really able to sire and raise high-quality offspring? This female selectiveness will force the male to elaborate even more on song and appearance in order to be picked by a female.

This ongoing process of manipulation and resistance drives the evolution of these sexual traits at a much faster rate in Eurasian Penduline-Tits than in Cape Penduline-Tits. In conclusion, their underlying breeding system may explain why some penduline-tit species exhibit a greater degree of sexual dimorphism than others, something that may hold true for a number of bird species.

The next step in our research will be to study other species of penduline-tits and see if sex differences in behaviour and morphology are generally related to their breeding system. In Central and East Asia, for instance, three species occur: the Black-headed, the Whitecrowned and the Chinese Penduline-Tit. They occupy diverse habitats, with varying quantities of food available and different climates, ranging from the Gobi Desert in China to reed-marshes in Kazakhstan. Investigating the environmental conditions may reveal some of the reasons why various breeding systems have evolved in penduline-tits. In addition through the use of DNA samples, we can assess the exact relationship between the species and may be able to reconstruct their phylogenetic history in order to discover which the ancestral species is: a dull, cooperative one or a bold macho?

*The research leading to these results received funding from the European Community's Sixth Framework Programme, the Hungarian/South African Intergovernmental TÉT, and the Schure-Beijerinck-Poppingfonds of the Royal Netherlands Academy of Sciences.*

## An unconventional hang-out

On 1 November 2006, I visited the farm Vaalkop in the Petrusburg district, approximately 80 kilometres south-west of Bloemfontein in the Free State, to check on a Secretarybird nest. The surrounding area of the farm is mainly *Themeda triandra* grassland, with shrubs concentrated on the hills and at the base of the hills. While driving through the open grassland on the secondary farm road, which is bordered on either side by a fence, I noticed the nest of a Cape Penduline- Tit on the third wire from the ground at a height of 1.15 metres. The nest was probably freshly built and was still empty. Seeds of the *Themeda* grass which had been used as nest material were also visible on the exterior of the nest.

On 15 November I revisited the site and saw that there were eggs in the nest, but it was unclear what the clutch size was (generally a clutch contains between four and seven eggs). On another visit to the nest, on 25 November, I inserted my finger into the nest to inspect it, and discovered an adult bird inside, brooding newly hatched young. A second clutch was also probably laid, as chicks were again present in the nest on 15 January 2007. A few days later, on 27 January, three nestlings were in the nest and ready to fledge.

This species attaches its nest to the branches of bushes at a height of one to three metres, the bushes acting as protection for the nest. The nest found at Vaalkop was approximately 1 000 metres or more from the hilly area or from the nearest bushes. While an explanation for this nest site on the fence could be that the *Themeda* grass there is long enough to provide some protection for the nest against predators, it was not really wind-resistant.

D. DE SWARDT

As far as I am aware, this is the first record for this species of a nest site on a man-made structure in an area away from its natural habitat of bushes and low shrubs.

**DAWIE DE SWARDT**

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# APPENDIX

# III

## PRACTICAL FIELD GUIDE FOR INVESTIGATING BREEDING ECOLOGY OF PENDULINE TITS *REMIZ PENDULINUS*

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*Photograph by C. Daroczi*

*Version 1.4* – 5 May 2009

# RATIONALE

*Why study penduline tits?* The main reason behind studying penduline tits is their extremely variable and among birds unusual breeding system: both sexes are sequentially polygamous. Both males and females may desert the clutch during the egg-laying phase, so that parental care is carried out by one parent only and, most remarkably, some 30-40% of clutches is deserted by both parents.

In this field guide we outline several methods that help us to reveal various aspects of the penduline tit's breeding system, including mate choice, mating behaviour, and parental care.

The motivation in writing this field guide is to guide you through a number of basic field methods and point your attention to some potential pitfalls. The penduline tit is a fairly easy species to study, but at the heart of unravelling its breeding ecology are appropriate, standardised and accurate field methods.

*Further reading*

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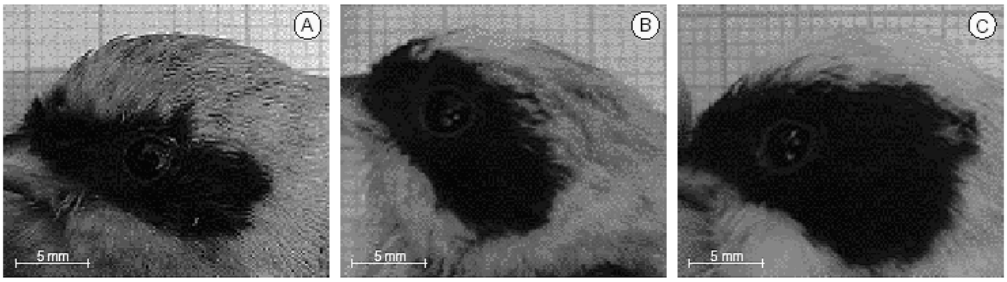
**Figure III.1** Typical habitat of penduline tits, dikes surrounding fishponds in Fehértó, Hungary. (photograph by T. Székely)

## THE PENDULINE TIT

*Male versus female*

The Eurasian penduline tit *Remiz pendulinus* is a small (body mass about 9g) passerine that breeds in reed marshes and gallery forests (Fig. III.1) throughout Europe and large parts of Asia. It is one of the 13 species currently classified within the subfamily of Remizinae (Harrap & Quinn 1996). Eurasian penduline tits are sexually dimorphic so that males and females can usually be sexed without much trouble in the field. Males are more brightly coloured than females: they have a dark red-brown mantle, reddish spots on the breast, bright white crown feathers and a wider eye-stripe (the 'mask') than females. Females are often paler (Fig. III.2). However, these differences are not always very clear (Fig. III.3); in ambiguous cases their behaviour may help, *e.g.* males sing, whereas females only call.

**Figure III.2** The male (left) is more brightly coloured and has a wider mask than the female (right). (photographs by R.E. van Dijk)




Female Male Male

**Figure III.3** The distinction between male and female is not always clear cut based on one trait, such as mask size. (photographs by S.A. Kingma)

*Nest*

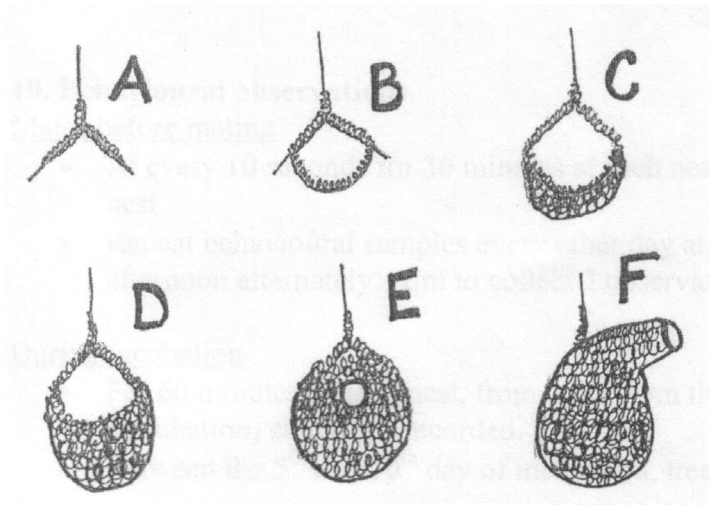
Penduline tits build elaborate, domed nests mainly from the pappus of reed *Phragmites australis*, poplar *Populus spp*. and willow *Salix spp*. (Fig. III.4). The nest building process can be divided into six stages (Fig. III.5). The male initiates the nest building process and may attract a female, usually from a stage 'C' or 'D' (see Fig. III.5). Male and female then jointly finish the nest. A male is considered 'mated' when the pair copulates near the nest, or when the male and the female build the nest together.

Nests in stage 'A' are usually initiated on the date the nest was found. Nests in stage 'B' are usually two days old and 'early' stage 'C' nests may be determined as three days old. The time to reach different stages from C onwards may vary over the breeding season. Early in the season, when few females are present, the male may stay in stage 'C' for weeks, later on a nest may be finished within a week.

*Eggs*

Once a nest is in stage 'E' the female often starts laying eggs, one per day, usually early morning. Once there are about three eggs in the nest, the male, the female, or both will desert the clutch. If they desert both, all investment in the current nest has been in vain and the nest will not be used again (although the material may be used for other nests). If the female deserts the male may stay behind to incubate the clutch and raise the offspring. Incubation is usually initiated one day after the female deserted. If the male deserts, the female may lay an additional number of eggs and only starts incubating once the clutch is completed. We consider a bird deserted if it has not been seen at the nest for at least two consecutive nest checks. The second check should last for at least 30 min (see *Chapter III*).

**Figure III.4** A nest in stage F of a Eurasian penduline tit. The colour-ringed male has just arrived on the right with some more nest material. At this stage the nest is likely soon to be deserted by either the male or the female. After desertion the caring parent, especially in case of female-only care, usually extends the spout a bit more. (photograph by R.E. van Dijk)




**A** – Small amount of material woven around the twig.
**B** – Ring. Bottom part very thin.
**C** – Basket, where both holes cover more than 50% of nest height
**D** – Bag, where both holes cover less than 50% of nest height
**E** – Nearly finished nest with one side closed, or at least not suitable as entrance/exit anymore; no spout yet
**F** – Finished nest. Spout may initially be not much more than a small 'roof'.

**Figure III.5** The major stages of nest building (drawing by I. Szentirmai).

During the egg-laying period the eggs may or may not be covered with nest material. We are unsure what the reason for this egg covering behaviour may be, but protection against cracks that may occur for instance due to strong wind could well

be a possibility (but see Valera et al. 1997). Keep this in mind when counting the eggs! NB sometimes eggs are buried very deeply in the bottom of the nest. Sometimes these eggs remain buried and are not incubated. Eggs are normally uncovered by the parent during the incubation phase. Eggs are normally counted on the eighth day of incubation, unless otherwise required for a specific project. Eggs can be counted by probing with your fingers inside the nest, supporting the bottom of the nest with the other hand. Do not take eggs out of the nest if not necessary. Also, only count eggs on predefined dates. Try to avoid any disturbance at all times! If the eggs were covered, restore the original situation after counting.

Also at the eighth day of incubation we usually take a three hour video-recording. The total period of incubation is 14-15 days.

*Nestlings*

Around the expected date of hatching, the actual hatching date should be determined. This should be done by observing the parent's behaviour: it will fly in and out much more frequently than during incubation and it will be carrying food items. Usually a slight hatching asynchrony (±1 day, but may sometimes be as many as three days) does occur, but, unless otherwise required, the first day of hatching is taken as the hatching date to avoid unnecessary disturbance.

At the tenth day after hatching (hatching date is day 0) a feeding frequency observation is performed by filming for three hours. After this recording the nestlings are ringed and measured, and a small blood sample (± 25µl) is taken. The chicks will fledge at around the 21st day after hatching.

**GENERAL FIELD METHODS**

At each nest visit write down the Date, Nest-site, Time, Bird present (male and/or female and colour-rings), briefly the behaviour of present bird(s), and any other useful comments. Also record GPS coordinates in UTM format for each nest and give a short and clear description of where the nest can be found (see Appendices I & II).

*Example*: 1D/2 – 512 (7.15) ♂ (MBOW) Bl, Ca, Si, BN; ♀ (ur) Bl, Ca. GPS: N107229 / E111078. Description: In second Poplar on the left after the lock, coming from 1N. Tree: Populus *sp*.

*Finding and checking nests*

Equipment needed: Notebook, GPS receiver, nest check notebook, binoculars, adhesive tape

(See Supplement IV for a satellite image of our study site Fehértó, Hungary)

We usually split the study area between fieldworkers. Make sure each part of the area is completely covered at least every other day. Walking over the dikes the birds can be located by their calls and songs, and/or movements. Once a bird caught your eye, the first thing to do is to look at its legs: Is it ringed, and if so, what is the colour-combination? Colour-codes are written down in the following order: Upper, under, left, right. So, a bird MBGO, has a Metal ring on top on its left tarsus followed by a Blue ring, and a Green ring on top on its right tarsus followed by an Orange ring. Colours we have or may use are: Yellow, Blue, White, Red, Green, Orange, Pink and black (the latter coded as 'S' from 'Swart', Afrikaans for Black).

To find its nest, you need to follow the bird and it will take you to its nest (if it has one). Each new nest should be labelled using an adhesive tape that should be attached to a tree or shrub 10m from the nest towards the field station (the latter to avoid helping other people finding the nests). Coding of nests works as follows: Each dike has its own unique ID. The first nest found on that dike is 1, the second 2, and so forth (e.g. 4D/10). The exact GPS coordinates (try to get as close to the nest as possible) should be taken for each nest using the UTM format. Record N and E coordinates. Every nest needs to be checked at least every other day during nest building; during incubation and feeding the nest check frequency may be somewhat lower, e.g. every fourth day. Fifteen minutes should be enough to record the presence of the bird(s) (see *Chapter III & IV*). The stage of the nest (Fig. III.5), tree species, birds present and their behaviour (briefly) should be recorded (see Supplement 1).

Biparentally deserted clutches should be taken to an incubator as soon as the nest has been recognised as 'biparentally deserted'.

(See Supplement II)

*Trapping Birds*

Equipment needed: set of poles, mist net, ropes, tent pegs to fix ropes, portable CD-player, two speakers, dummy penduline tit, old nest, cotton-wool, bird bags

- Do not try to trap a nest building male before an advanced stage C, otherwise the bird will very likely abandon its nest.
- Do not try to trap a pair on the day they got mated, otherwise the female is likely to abandon her mate.
- Do not try longer than 30min at all times. If you cannot catch the bird in 30min, you very likely cannot catch it in one hour either, i.e. waste of time and unnecessary disturbance risking abandonment.
- Do not try to trap during rainfall. These small birds easily cool down and die because of that.
- Do not use the 'Barbácsy's trap' (see Fig. III.6) before the eighth day of incubation, to avoid unnecessary abandonment.
- Make sure you always carry a pair of (sharp) scissors with you. If you do not manage to free the bird within reasonable time, some careful cuts in the net may help. But be patient; penduline tits are usually not the most difficult to release from the net. Ask for help of more experienced researchers if so required.
- Try to work quick and efficient. If possible, go trapping together.

Ideally both male and female should be trapped together during the period that they are together using a mist net:

- Position the mist net not too far from the nest, and preferably in the shadow. Avoid the wind as much as possible. Try to remember the bird's behaviour: Many birds use their favourite passages when leaving the nest site. Set up the net just there if possible.
- Set up the net as high as possible. Make sure there are no branches above or close to the net, where the bird can take its time to sit on and thoroughly inspect what is going on here.
- On the opposite side of the net (than the nest) a portable CD-player should be placed playing penduline tit song. Right above the speakers an old nest, cotton-wool, and a dummy penduline tit should be hung in a (artificial) tree.

**Figure III.6** The Barbácsy's trap. (photograph by R.E. van Dijk)

If you do not manage to trap the bird within 30 min., try again the next day. Unsuccessful again? Then skip one day and try again on day four.

It is of no use to try and trap with the above set up during incubation. Trapping the birds at their nest using the 'Barbácsy's trap' is much more successful, but wait using this trap until day eight of incubation. Also feeding birds may be trapped using 'Barbácsy's trap'.

*Handling Birds*

Equipment needed: metal rings, ringing plier, colour-rings, colour-ring clip, colour-ring table, digital calliper, ruler, digital camera, gray card, Pesola spring balance, yellow notes, waterproof pen, needles, capillaries, cotton-wool, Eppendorf tubes, capture notebook

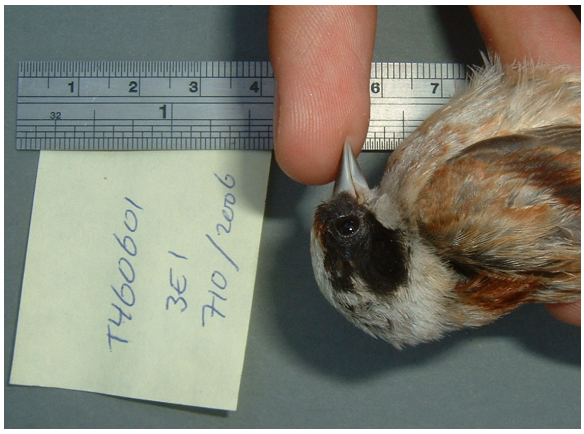



**Figure III.7** When you take a photo of the mask of a bird, make sure the gray card, the ruler and a note containing date, nest site and ring number are clearly visible. (photograph by R.E. van Dijk)

- The bird's health is more important than collecting all data. For instance, do not try excessively long to get some blood sample. If it does not work within a reasonably time, move on.

Ringing, measuring and sampling blood (in order in which they should be carried out):

- Metal ring, on the left tarsus. Make sure the ring can be read easily when one holds the bird on its back in the hand (*i.e.* not upside down).
- Measure left tarsus.
- Colour-rings. Make sure you get the order right.
- Wing length. Outer primaries in straight line along the ruler of right wing.
- Fat score. In five classes in furcular depression (in throat just above sternum).
- Mask photos (Fig. III.7). Three photos from each side of the head. Make sure the ruler, ring number, AND gray card are visible. The bird should touch the gray card with its shoulder. Make all photos of the bird and gray card in the shade.
- Photos of back and neck. Make sure the gray card is visible (in similar light conditions as the bird).
- Body mass. Make sure the Pesola spring balance is calibrated to 0g with bag.
- Blood sampling. Take small (± 25µl, 1-2cm in a capillary) sample from the brachial vein. Store blood in labelled (Ring number, species, sex, date, year) Eppendorf tube containing Queen's lysis buffer (Seutin et al. 1991). Use Leukoplast as a label, do not write on the side of the tube, this will easily wear off. Write on the leukoplast label with waterproof pen. Copy the label, or at least ring number on the lid. Make sure the lid is properly closed. You may want to seal the lid using, for instance, parafilm wrapped around it to ensure it will not open whilst travelling. Refrigerate blood a.s.a.p. (Fig. III.8).

**Figure III.8** Blood sampling. (photograph by R.E. van Dijk)

(See Supplement III)

*Fates of nests*

Nest fates are usually scored using the following scheme:

ABAND – nest is abandoned by the male during nest building (before pair formation)

CLABAND – clutch abandonment. Caring parent abandoned clutch (or nestlings). Often due to some kind of disturbance

DES – biparental desertion. Both male and female deserted after pair formation, before incubation

DEST – destroyed. Usually by the wind, sometimes by humans.

FLED – 'fledged'. Defined as presence of nestlings at day 10 after hatching.

PRED – predation. Nest is predated. Usually during nestling phase, nest partly destroyed.

TAKEN – nest is overtaken by another male. Usually happens during nest building phase. Nest code will remain the same, but indicated as 'a' and 'b' (*e.g.* 4D/10a and 4D/10b)

**********DISCLAIMER**********

*Please note that the authors are not liable for any consequences of the use (or* *misuse) of this guide. You need to check regulations and legislation in the* *country and site where you are carrying out the field work. Also make sure you* *are aware of the health and safety instructions as provided by your institution, and* *take any potential risk or hazard that accompanies your fieldwork seriously. We* *did not deal with essential conceptual and practical issues for successful* *fieldwork (e.g. experimental design and logistics): each particular project and* *experiment requires its own innovative ideas. We will always be happy to discuss* *ideas, and welcome suggestions to improve the study of penduline tits.* *Good luck! *

**********

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***SUPPLEMENT I.*** Behavioural codes of penduline tits at the nest.

***Frequent behaviour***

| Behaviour | Explanation | Abbreviation |
|---|---|---|
| Away | not present within a 10m radius around the nest | A |
| Building nest | | BL |
| Calling | | CA |
| Calling from inside nest | | CAN |
| Cleaning Bill | | CB |
| Covered | present within 10m radius, but invisible / covered by leaves, branches, etc | COV |
| Feeding | foraging | FE |
| Flying | | FL |
| Gathering nest material | | GN |
| Hanging on the nest | | HN |
| Incubating | | I |
| Perching | sitting somewhere resting / doing nothing | P |
| Preening | cleaning feathers | PR |
| Sitting in the nest | doing nothing | SN |

***Infrequent behaviour***

| Behaviour | Explanation | Abbreviation |
|---|---|---|
| Bringing nest material | to the nest | BN |
| Chasing | some bird; write down details | CH |
| Chasing away female | | CHF |
| Chasing away intruder | unknown enemy PT; write down details of intruder: rings, male or female | CHI |
| Chasing away male | | CHM |
| Chasing away mate | | CM |
| Copulation | | CO |
| Fighting | with unknown enemy | FI |
| Fighting against female | | FIF |
| Fighting against male | | FIM |
| Fighting against mate | | FM |
| Nest material delivery | male delivers material to building female | NMD |
| Singing | | SI |
| Singing from inside the nest | | SIN |
| Singing on outside of nest | bird is hanging on the nest and singing | SON |
| Soliciting copulation | female is flapping her wings | SC |
| Trying to enter nest | male tries to enter the nest, but female blocks entrance | TE |

***SUPPLEMENT II.*** Nest records. The spreadsheet containing the nest data looks like this. Make sure you collect these for each nest.

| YEAR | SITE | NEST | MALE CODE | FEMALE CODE | FOUND | FOUNDST | ECOORD | NCOORD | TREE | STBUILD | MATDAT | DESPAR | DESM | DESF | EGGS | CH10 | FATE | END | DIST | OBS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2007 | 4D | 11 | MOYG | YYWM | 607 | A | 733201 | 107936 | Salix | 607 | 614 | M | 620 | | 5 | 4 | FLED | 728 | 0 | RD | |
| 2007 | CH | 10 | BRWM | BBMO | 601 | D | 734210 | 117543 | Populus | 523 | | | | | | | ABAND | 607 | 0 | LM | |
| 2007 | FT | 6 | WWWM | YMOR | 526 | C | 741443 | 109856 | Salix | 521 | 528 | MF | 601 | 601 | 3 | | DES | 601 | 0 | ZG | |
| 2007 | 1D | 8 | YYRM | BMOG | 701 | B | 729043 | 110998 | Salix | 630 | | | | | | | ABAND | 702 | 1 | RD | tre |

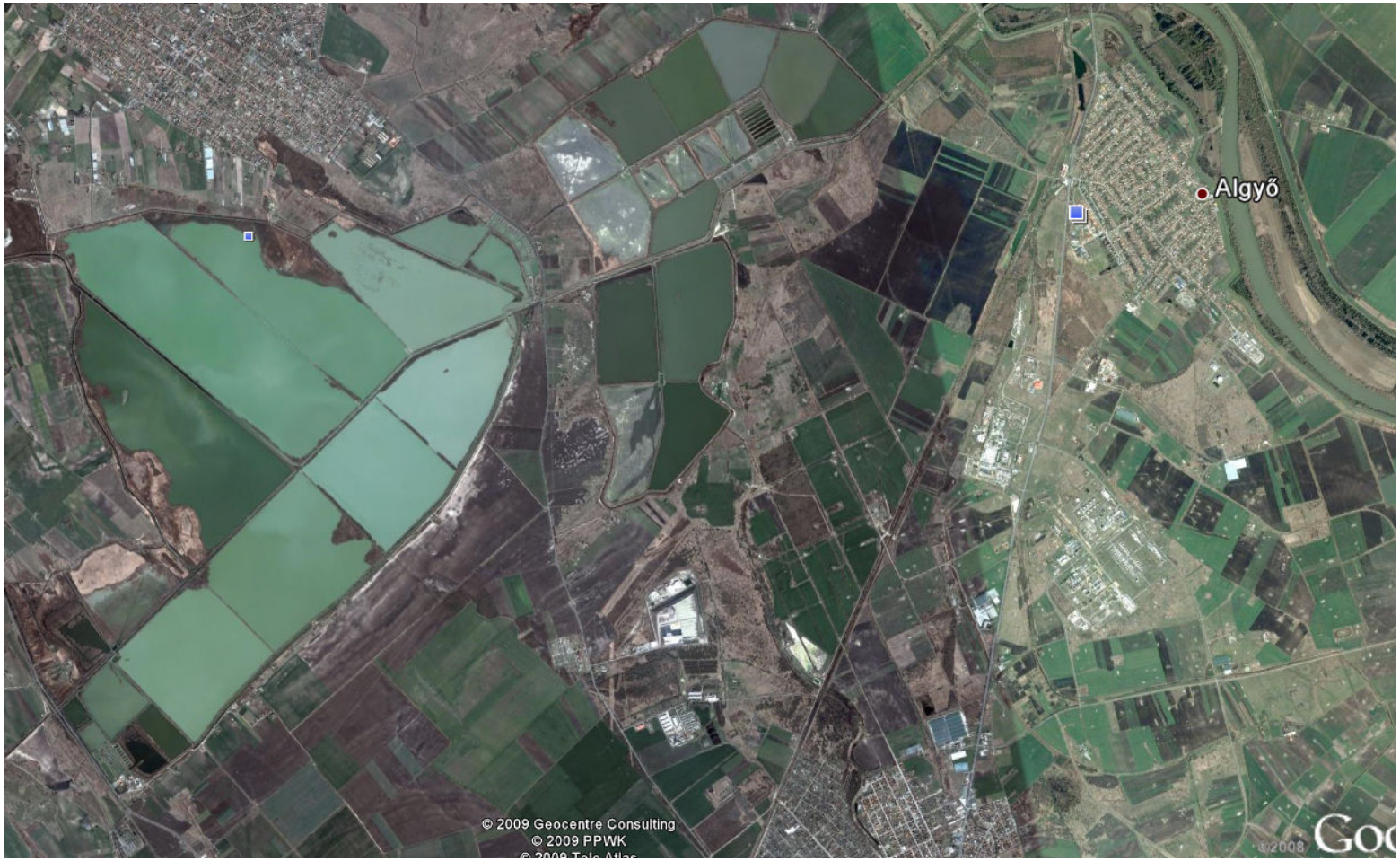
**YEAR**
**SITE** - Each dike or patch has its unique 'site code'
**NEST** - Give consecutive numbers for each nest found for a given site in the order in which they were found.
**MALE CODE** - Rings of the male
**FEMALE CODE** - Rings of the female
**FOUND** - The date at which the nest was found
**FOUNDST** - The stage in which the nest was found (see Fig. 3)
**ECOORD** - East GPS Coordinates of the nest, following UTM format
**NCOORD** - North GPS Coordinates of the nest, following UTM format
**TREE** - The tree in which the nest is built
**STBUILD** - Date of nest building initiation
**MATDAT** - Date of pair formation
**DESPAR** - Deserted parent
**DESM** - Date of desertion by the male
**DESF** - Date of desertion by the female
**EGGS** - Number of eggs at the eighth day of incubation
**CH10** - Number of nestlings at the tenth day after hatching
**FATE** - Fledged, deserted, abandoned, etc.
**END** - Last date when the nest was checked
**DIST** - Was the nest disturbed? 1=yes, 0=no
**OBS** - Observer
**COMM** - Comments

***SUPPLEMENT III.*** Capture records. The spreadsheet containing the capture data looks like this. Make sure you collect these for captured birds.

| RING | CODE | YEAR | DATE | TIME | SITE | NEST | SEX | WING | TARSUS | FAT | BROOD | MOULT | WEIGHT | BLOOD | PHOTO_L | PHOTO_R | PHOTO_HB | OBS | COMM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7E7431 | MBGR | 2007 | 620 | 9:15 | CH | 10 | M | 56 | 17.2 | 3 | 0 | 0 | 9.25 | OK | OK | OK | OK | RD | |
| 7E7556 | GYRM | 2007 | 617 | 12:20 | 1D | 9 | F | 57 | 17.0 | 4 | 1 | 1 | 11.25 | OK | OK | OK | OK | LM | |
| 7E7332 | YMBR | 2007 | 712 | 16:30 | 3E | 1 | JUV | 25 | 16.7 | 5 | | | 9.75 | OK | | | | LM | |

**RING** - Metal ring number
**CODE** - All rings
**YEAR**
**DATE** - Date of capture
**TIME** - Time of capture
**SITE**
**NEST**
**SEX** - M = male, F = female, Juv is juvenile (a juvenile trapped in mist net may not necessarily belong to the nest where the net was set up)
**WING** - Wing length as measured with stretched primaries of right wing (mm)
**TARSUS** - Tarsus length of left tarsus (to 0.1 mm)
**FAT** - Five classes in furcular depression
**BROOD** - Brood patch present (1) or not (0)?
**MOULT** - Is the bird moulting (1) or not (0)?
**WEIGHT** - Body mass (g)
**BLOOD** - Is a small blood sample taken (OK)?
**PHOTO_L** - Is a photo taken of the left mask (OK)?
**PHOTO_R** - Is a photo taken of the right mask (OK)?
**PHOTO_HB** - Is a photo taken of the head and back (OK)?
**OBS** - Observer
**COMM** - Comments

***SUPPLEMENT IV.*** Satellite image of Fehértó, Hungary. Clearly visible are the large fishponds surrounded by dikes. On the right the River Tisza, which may host many penduline tits in its gallery forest (from Google Earth).



# APPENDIX

## IV

*The Evolution of Breeding Systems and the Impact of Sexual Conflict and Cooperation in Penduline Tits*

## FIELDWORK REPORT

## EXPEDITION KAZAKHSTAN 2008

*18 May – 29 June*

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*Photographs by R.E. van Dijk*

23 June 2008

## Members of the expedition team penduline tits in Kazakhstan:

*From left to right*: Sander Bot (University of Groningen, The Netherlands), Vera Voronova (University of Karaganda, Kazakhstan), René E. van Dijk (University of Bath, UK)

### OBJECTIVES

1. To describe the breeding system of a. White-crowned penduline tits *Remiz coronatus*, and b. Black-headed penduline tits *Remiz macronyx*
2. To investigate to what extent habitat can predict levels of cooperation and conflict in penduline tits
3. To investigate the impact of conflict and cooperation on the evolution of morphological traits
4. To investigate the impact of conflict and cooperation on the evolution of behavioural traits
5. To reconstruct a phylogenetic tree of penduline tits (blood sampling, aimed sample size: 40)

### HYPOTHESES & PREDICTIONS

**H1.** The abundance of food and nest material influences the level of cooperation in breeding

**P1.** Black-headed penduline tits, which occur in allegedly food rich reed beds, exhibit higher levels of conflict, including uniparental care and polygamy, than

White-crowned penduline tits, which live in the relatively poor foothills of the Tien Shan mountains (biparental care and monogamy)

**H2.** The level cooperation influences morphology and song via an ongoing process of manipulation and resistance associated with sexual conflict

**P2.** The species exhibiting more intense sexual conflict will show a more intense sexual plumage dimorphism and the male will have a more complex song than the species with lower levels of conflict

**H3.** The level of cooperation in breeding system is reflected in parental behaviour

**P3.** The species exhibiting more intense sexual conflict will i. attend the nest less frequently, and ii. attend the nest less synchronously.

Central Asia may be the cradle of the subfamily Remizinae. Our phylogenetic tree may reveal this. The ancestral breeding system of the polygamous Eurasian penduline tits *Remiz pendulinus pendulinus*, with habitat characteristics and various morphological traits superimposed on the phylogeny may provide important insight into the evolution of the diverse breeding systems within the relatively confined group of penduline tits.

## METHODS

We visited two field sites in Kazakhstan: for the White-crowned penduline tits the foothills of the Tien Shan mountains near Jabagly (42°25'N, 70°29'E) (10 May – 10 June), for Black-headed penduline tits the Topar Lakes, near Topar (45°02'N, 75°01'E) (12 – 26 June) (see Fig. IV.1).

Default protocol field methods for penduline tit research, including monitoring of incubation behaviour, were used (see *Appendix III*). Additionally, nest attendance was filmed during the period of egg-laying using the Sony DCR-HC44E digital camcorder, over full daylight periods at a resolution of one frame per 5 seconds (see *Chapter IV and IX* for further details on field methodology). Due to the inaccessibility of most nests of the White-crowned penduline tits at Jabagly, the exact phase of egg-laying was not known, but nests were filmed from a stage E of nest building onwards (see *Appendix III*), which, at least in Eurasian penduline tits, is when egg-laying takes place. One nest of a Black-headed penduline tit pair (*R. m.*

*ssaposhnikowi*) was monitored for nest attendance at exactly the correct period, at two and three eggs.

Penduline tits are not resident in Kazakhstan, but migrate southwards during winter.

(A)

(B)

**Figure IV.1** (A) The foothills of the Tien Shan mountain range at Jabagly. The arrow indicates one of the valleys in which the White-crowned penduline tits build their nests, mainly on hawthorn trees (*Crataegus sp.*). Four of these valleys, average distance between them about 2.7km, were included in our research. (B) A typical habitat of Black-headed penduline tits, reed beds along the Topar Lakes, near Topar.

## RESULTS

*White-crowned penduline tit*

(In this report the White-crowned penduline tit is the subspecies *R. c. coronatus*)

Habitat

The habitat of the White-crowned penduline tit near Jabagly consisted of, mainly, hawthorn (*Crataegus sp.*) trees with a few willows (*Salix sp.*) and poplars (*Populus sp.*) aligning small streams coming down from the Tien Shan mountains through the foothills. The penduline tits build their nests in these trees, and use various, yet typical, materials for their nests - material which appears to be scarcely available. The limited amount of vegetation (beyond the aforementioned trees and some shrubbery, there is not much more than open grassland) results in that food resources are rather poor. Although this requires some measure of quantification (*e.g.* NDVI LandSat images), it seemed to us very much less available than in, for example, our field site in Hungary, Fehértó.

The habitat of the White-crowned penduline tits at Topar was rather different, yet with regards to the amount of food and nest material comparable to Jabagly. At Topar their habitat was a small side river of the river Topar, running through the sand dunes. Along this river some reed (*Phragmites australis*) and bulrush (*Typha sp.*). The nests were built in Russian olives (*Eleagnus angustifolius*). Immediately beyond these narrow reed beds semi-desert. The high density of nests of White-crowned penduline tits here was remarkable.

Biometrics and song

(A) (B)

**Figure IV.2** Male (A) and female (B) White-crowned penduline tit *Remiz coronatus*.

White-crowned penduline tits have a sexually dimorphic plumage, the extent to which has yet to be analysed: Males generally have a wider mask than females which may extend onto the nape, sometimes only leaving a white crown. Males are also more brightly coloured with darker mantle and wing coverts, and whiter crown than females (Fig. IV.2A). Females may have black on the nape too (Fig. IV.2B). A characteristics feature of the White-crowned penduline tits plumage compared to Eurasian and Black-headed penduline tit is the complete lack of red-brown feathers on the head and breast.

White-crowned penduline tits are smaller and lighter than Eurasian penduline tits (mean ± SD: 7.36 ± 0.48g versus 9.62 ± 0.87g). Females (7.72 ± 0.52g) seem slightly heavier than males (7.14 ± 0.27g), as usual.

Song seems very similar to Eurasian penduline tits: different syllables are used, but complexity appears not to differ very much from the Eurasian penduline tits. This, however, requires detailed analyses. We were clearly more successful in mistnetting the birds using a playback of White-crowned penduline tit song than with a playback of the song of Eurasian penduline tits.

Parental care

Nests are built by male and female together from stage A onwards, although an unmated male will start on its own (Fig. IV.3). Sometimes the pair seems to work closely together, mostly in earlier stages of nest building, but in general they appear to work pretty much 'independently' of one another (in contrast to the cooperative Cape penduline tits *Anthoscopus minutus*; see *Chapter IX*).

Of the **18** nests where we observed parental care, **incubation** was **always** carried out by **male and female. However**, at **4** nests the young were **fed** by **only one parent, 2 male-only and 2 female-only**. Additionally, Yevgeni Belousov had observed a feeding female-only in the same season in Aksu-Jabagly National Park.

**Clutch size** was determined at 4 nests: **6.75** ± 2.63 eggs.

Number of **nestlings** was determined at 6 nests (at various ages): **5.83** ± 2.32 nestlings.

**Mate switching** has not been observed.

At **3** nests the **clutch** was **abandoned** during incubation.

**Predation** took place at **1** nest (possibly at 2 nests).

Summary of collected data

| | |
|---|---|
| Nests: | **32** (25 at Jabagly, 7 at Topar) |
| Trapped males: | **22** (19 at Jabagly, 3 at Topar) |
| Trapped females: | **14** (13 at Jabagly, 1 at Topar) |
| Sampled nestlings: | 31 (all at Jabagly) |

All individuals trapped (N = **67**) have been sampled for blood (two of which may not contain enough blood for analysis).
All trapped adults have been photographed for mask size and saturation of back.

| | |
|---|---|
| Process video: | **7** pairs (all at Jabagly; 5 of which for one day only) |
| Song recording: | **12** males (all at Jabagly) |
| Incubation video: | **10** nests (9 at Jabagly, 1 at Topar) |

**Figure IV.3** Male White-crowned penduline tit at its nest

*Black-headed penduline tit*

(In this report the subspecies *R. m. macronyx* and *R. m. ssaposhnikowi* and any hybrids are lumped under this name, unless otherwise stated)

Habitat

The habitat where we have been searching for Black-headed penduline tits consisted of semi-desert interspersed with small lakes and marshes. The relatively small lakes in these sand dunes apparently contained too little vegetation for penduline tits. It also seemed rather dry containing very little food. Black-headed penduline tits were to be found in more extensive reed beds close to the river Topar (at many places inaccessible) (Fig. IV.1B). The *ssaposhnikowi* subspecies built its nest in a tree (a Russian olive), although black-headed penduline tits may build their nests in reeds and are thus likely less dependent on trees than, for instance, White-crowned penduline tits. Food resources in these reed beds were plentiful. Nest material too was much more abundant then at Jabagly mainly due to the abundance of reed and bulrush.

Biometrics and song

Black-headed penduline tits have a clearly sexually dimorphic plumage, the extent to which has yet to be analysed:

*ssaposhnikowi*: Males have a black mask, a chestnut brown crown and nape, and a whitish throat (very much like *Remiz pendulinus caspius*). A deeply coloured dark red-brown mantle and wing coverts, and a lot of red colouration on the breast (Fig. IV.4A). Females are very much like Eurasian penduline tit males, yet with typical female characteristics: They have a wide mask, but squared rather than conically shaped and interspersed with some grey feathers, in particular at the base of the bill. A grey ring around the eye. A clear red fringe on the head above forehead patch extending to both sides of the crown. Lots of red feathers on the breast. The back is paler than the males (Fig. IV.4B).

*macronyx*: Males have a completely black head, a very dark reddish breast and red-brown mantle (Fig. IV.4C). Females have not been observed in our field (but see Fig. IV.4D).

Black-headed penduline tits appear to be of similar size and weight as Eurasian penduline tits (mean ± SD: 10.75 ± 0.64g versus 9.62 ± 0.87g). Females (11.20g) seem slightly heavier than males (10.30g), as usual.

Song (and call) is clearly different from Eurasian penduline tits: different syllables are used, at a slightly different frequency, but complexity may not be very different. This, obviously, requires detailed analyses.

Parental care

The two A-stage nests that we found were built by single males. The other nest, found in stage D, was attended by the pair when we found it. Again, the pair seems to work pretty much independently from each other and cannot be observed at the nest together very often.

At the one nest where we have been able to observe incubation, this was **female-only care**. The male deserted when there were three eggs in the nest: VERY similar to Eurasian penduline tit *Remiz pendulinus pendulinus*. (The eggs were initially covered, and uncovered on the day the male had deserted (Valera et al. 1997)).

Additionally, Black-headed penduline tits in Turkmenistan (*Remiz macronyx neglectus*) appear to exhibit female-only care too. The male has never been observed to take up parental care, nor seems biparental desertion to make up part of the breeding system (Y.M. Belousov, pers. comm.). The male in this species is described to desert at 2 eggs (Belousov 1979).

**Clutch size** was determined at 1 nest: **6 eggs**

We have not been able to determine the number of nestlings of Black-headed penduline tits.

Summary of collected data

| | |
|---|---|
| Nests: | **3** (two of which in stage A after which they were abandoned) |
| Trapped males: | **1** (possibly hybrid *macronyx* X *ssaposhnikowi*) |
| Trapped females: | **1** (subspecies *ssaposhnikowi*) |
| Sampled nestlings: | 0 |

All individuals trapped (N = **2**) have been sampled for blood.

All trapped adults have been photographed for mask size and saturation of back.

| | |
|---|---|
| Process video: | **1** pair (*ssaposhnikowi* pair) |
| Song recording: | **1** male |
| Incubation video: | 0 nests |

(A) 

(B) 

(C) 

(D) 

**Figure IV.4** (A) Male *R. m. ssaposhnikowi*. (B) Female *R. m. ssaposhnikowi*. (C) Male Black-headed penduline tit, possibly a hybrid *ssaposhnikowi* x *macronyx* since the amount of red-brown on the head and white on the throat patch is markedly less than in a typical *ssaposhnikowi* male (see (A)). (D) Male (top) and female (bottom) Black-headed penduline tit *R. m. neglectus* from Turkmenistan (courtesy photo Y.M. Belousov).

In total we have seen:

4 *R. macronyx macronyx* males

4 *R. m. ssaposhnikowi* males

2 *R. m. ssaposhnikowi* females

3 *R. pendulinus*-like females, which likely have been of subspecies *ssaposhnikowi.*

The population of penduline tits at the Topar Lakes thus consists of at least three different (sub)species, including the White-crowned penduline tits *R. c. coronatus*.

## CONCLUSIONS

1. Although we have limited data on the Black-headed penduline tits, we can fairly confidentially describe the breeding system of both species: The White-crowned penduline tits exhibits biparental care (NB feeding may be uniparental in some cases), whereas the Black-headed penduline tit seems to exhibit uniparental care (in accordance with observations from Turkmenistan (Y.M. Belousov, *pers. comm.*)), *i.e.* two radically different breeding systems within Kazakhstan. Detailed description (as to the exact share in incubation between male and female, for example) will follow from analyses of videos.

2. Importantly, the predicted relation between breeding habitat and breeding system IS supported: rich habitats were associated to less cooperation and a thus more polygamous breeding system; poor habitats were associated to cooperation resulting in biparental care.

3, 4. From observations in the field we note that both species exhibit sexual dimorphism, although much stronger in the polygamous Black-headed penduline tit. How the plumage dimorphism, song complexity, and behaviour fit the predictions in relation to conflict and cooperation remains to be analysed. We have collected a substantial amount of data to further investigate this.

5. We have collected a sufficient amount of blood samples to be able to reconstruct a phylogenetic tree of penduline tits. DNA samples collected from other populations/species will be included (i.e. *Remiz consobrinus consobrinus, Remiz coronatus stoliczkae, Remiz pendulinus pendulinus,* and *Anthoscopus minutus gigi*; Supplement A). Ideally, also DNA from Verdin *Auriparus flaviceps* and possibly the Fire-capped tit *Cephalopyrus flammiceps* and Tit-hylia *Pholidornis rushiae* will be included. DNA of the Verdin has been requested from prof Robert Zink, University of Minnesota. Alternatively, Clemens Kuepper, University of Bath, may be able to get DNA from subspecies *A. f. sinaloae*. A number of sequences for *Auriparus flaviceps* are available from GenBank (COI, cytb, RAG1, and mtDNA). The Fire-capped tit and Tit-hylia seem rather elusive. Hopefully some museum specimens, for instance, may be a solution.

**ACKNOWLEDGEMENTS**

I am grateful to Sander Bot (University of Groningen, The Netherlands) and Vera Voronova (University of Karaganda, Kazakhstan) for their important contribution to the fieldwork. I am thankful to Lammert Bies, Sergey Sklyarenko, Machiel Valkenburg, and Arend Wassink for their essential support in arranging practicalities. I also thank Yevgeni Belousov for sharing the information he collected in the '70ies on *R. m. neglectus*. The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002-2006) under contract n. 28696 (GEBACO and INCORE). Further financial support came from a University of Bath Studentship to REvD.

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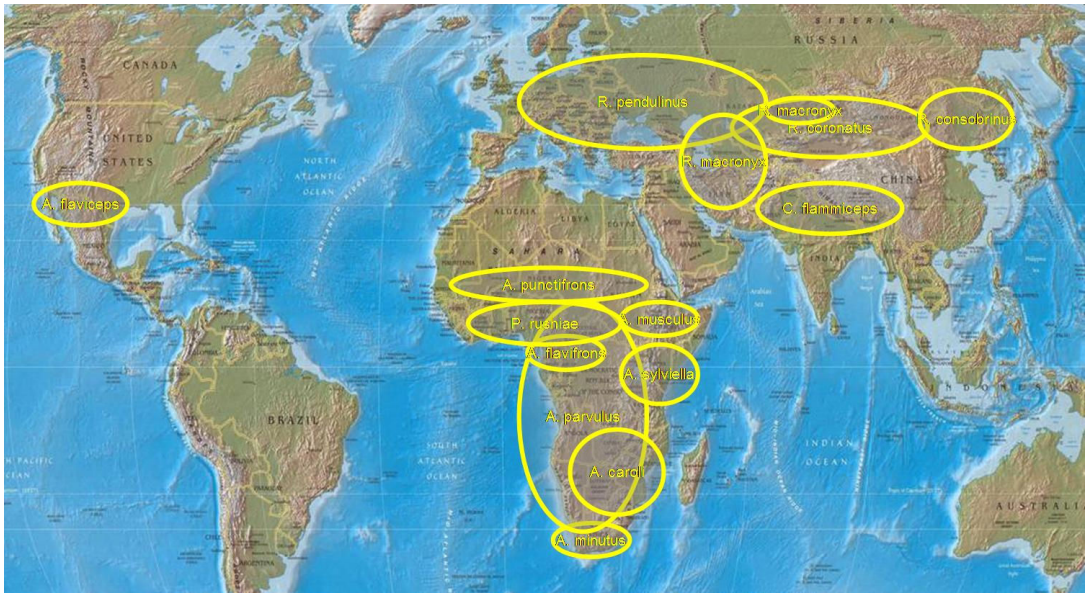
Jabagly

## SUPPLEMENT A




Distribution of all 14 penduline tit species of the world (NB *Anthoscopus sylviella* is not mentioned in Harrap & Quinn 1996; *Remiz macronyx* is not given the species status by Sibley & Monroe 1993). Noteworthy are *Auriparus flaviceps, Cephalopyrus flammiceps,* and *Pholidornis rushiae* given their 'odd' distribution and slightly different (nest building) behaviour.

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